10/070489

JC10 Rec'd PCT/PTO 01 MAR 2002

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I hereby certify that this paper of fee and the papers indicated as being attached hereto are being deposited with the United States Postal Service "Express Mail Post Office to Addressee" Service under 37 C.F.R. §1.10 on the date indicated above and are addressed to the Commissioner of Patents and Trademarks, P.O. Box 2327, BOX PCT, Arlington, VA 22202.

Alicia Bradbury
(Typed or printed name of person mailing)

Alicia Bradbury
(Signature of person mailing)

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. §371		ATTORNEY DOCKET NUMBER 24747-1104US
INTERNATIONAL APPLICATION NO. PCT/NZ00/00174	INTERNATIONAL FILING DATE 04 September 2000	PRIORITY DATE CLAIMED 02 September 1999

TITLE: **NUCLEOTIDE SEQUENCES ENCODING AN INSECTICIDAL PROTEIN COMPLEX FROM SERRATIA**

APPLICANTS FOR DO/EO/US: Travis Robert Glare, Mark Robin Holmes Hurst, Trevor Anthony Jackson

Applicant herewith submitsto the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. §371.
2. ☐ This is a **SECOND OR SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. §371.
3. ☒ This express request to begin national examination procedures (35 U.S.C. §371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. §371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. §371(c)(2)):
   a. ☒ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ A translation of the International Application into English (35 U.S.C. §371(c)(2)).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. §371(c)(3)):
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. §371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. §371(c)(4)).
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. §371(c)(5)).

Items 11 to 16 concern other documents or information included:

11. ☐ An Information Disclosure Statement under 37 C.F.R. §§1.97 and 1.98.
12. ☐ A DECLARATION and POWER OF ATTORNEY with claim under 35 U.S.C. §119 for benefit of priority to Application Serial No. New Zealand Patent No. 337610 will be submitted
13. ☒ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☒ ☐ Other items of information:
    A SEQUENCE LISTING and DISK copy thereof with Verified Statement.

JC13 Rec'd PCT/PTO 01 MAR 2002

APPLICATION NO.	INTERNATIONAL APPLICATION NO.	ATTORNEY DOCKET NUMBER
10/070489	PCT/NZ00/00[illegible]74	24747-1104US

17. [X] The following fees are submitted:		CALCULATIONS	PTO USE ONLY
Basic National Fee (37 CFR 1.492(a)(1)-(5)): Search Report has been prepared by the EPO or JPO	$ 890.00		
International preliminary examination fee paid to PTO (37 CFR 1.482)	$710.00		
No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2))	$ 740.00		
Neither international preliminary examination fee paid to USPTO (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO	$1040.00		
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Articles 33(2)-(4)	$ 100.00		
ENTER APPROPRIATE BASIC FEE AMOUNT =		$ 890.00	
Sur**c**harge of **$130.00** for furnishing the oath or declaration later than [ ] 20 [X] 30 months for the earliest claimed priority date (37 CFR 1.492(e)).		$ 130.00	

Claims*	Number Filed	Number Extra	Rate		
Total claims	48	28	18	$ 504.00	
Independent claims	1	0	84	$ 0.00	
Multiple dependent claims		0	280	$ 0.00	

* **BASED UPON ENTRY OF THE ATTACHED PRELIMINARY AMENDMENT** **TOTAL OF ABOVE CALCULATIONS =**	$ .00	
Reduction by 1/2 for filing small entity	.00	
SUBTOTAL =	$ .00	
Processing fee of **$130.00** for furnishing the English translation later than [ ] 20 [ ] 30 months for the earliest claimed priority date (37 CFR 1.492(f)).	$	
TOTAL NATIONAL FEE =	$1,524.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment accompanied by an appropriate cover sheet (37 CFR 3,28, 3.31) $40.00 per property.	$ 0.00	
TOTAL FEES ENCLOSED =	$1,524.00	
	Amount to be: refunded	$
	charged	$

a. [X] A check in the amount of $1,524.00 to cover the above fees is enclosed. A duplicate of this sheet is enclosed.

b. [X] Please charge Deposit Account No. 50-1213 for the above fees or for any amount due that is not covered by the enclosed check or if the enclosed check is in the wrong amount, post-dated or otherwise improper. A duplicate of this sheet is enclosed.

c. [X] The Commissioner is hereby authorized to charge any other fees that may be required, or credit any overpayment to Deposit Account No. 50-1213 is enclosed.

SEND ALL CORRESPONDENCE TO:

Stephanie Seidman

Stephanie Seidman
Heller Ehrman White & McAuliffe LLP
4350 La Jolla Village Drive, 7th Floor
San Diego, CA 92122
Telephone: (858) 450-8400
Facsimile: (858) 587-5360

10/070489

Rec'd PCT/PTO 17 SEP 2002

#5

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Glare *et al.*

Serial No.: 10/070,489

Filed: March 1, 2002

For: *NUCLEOTIDE SEQUENCES ENCODING AN INSECTICIDAL PROTEIN COMPLEX FROM SERRATIA*

Confirmation No.:6955

Art Unit: Unassigned

Examiner: Unassigned

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Date of Deposit September 17, 2002

I hereby certify that this paper is being deposited with the United States Postal "Express Mail Post Office to Addressee" Service under 37 C.F.R. §1.10 on the date indicated above and addressed to:

Commissioner for Patents
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P.O. Box 2327
Arlington, VA 22202, on this date.

09/17/02 Kimila Carraway

Date Kimila Carraway

AMENDMENT IN RESPONSE TO NOTICE TO COMPLY WITH REQUIREMENTS FOR PATENT APPLICATIONS CONTAINING NUCLEOTIDE SEQUENCE AND/OR AMINO ACID SEQUENCE DISCLOSURES

Box Missing Parts
Commissioner for Patents
U.S. Patent and Trademark Office
P.O. Box 2327
Arlington, VA 22202

Dear Sir:

Responsive to the Notice to File Missing Parts of Nonprovisional Application and the Raw Sequence Listing Error Report, mailed June 19, 2002, please amend the application as follows:

IN THE SEQUENCE LISTING:

Please replace the sequence listing in the above-captioned application with the attached replacement SEQUENCE LISTING. A disk copy of the SEQUENCE LISTING accompanies this response.

REMARKS

A check for the fee for a one month extension of time accompanies this response. The Commissioner is authorized to charge any additional fee that may be due in connection with this paper or with this application during its entire pendency may be charged to Deposit Account No. 50-1213. If a Petition for extension of time is needed, this paper is to be considered such Petition.

USSN 10/070,489
Glare *et al.*
AMENDMENT IN RESPONSE TO NOTICE TO COMPLY

Attached herewith is a copy of the Notice to File Missing Parts of a Nonprovisional Application mailed June 19, 2002 and the Raw Sequence Listing Error Report, paper and disk copies of the replacement Sequence Listing, and a Verified Statement that the content of the paper and computer readable copies are the same.

The replacement Sequence Listing differs from the Sequence Listing as originally filed in that the replacement Sequence Listing is prepared in FastSEQ for Windows Version 4.0 and reflects corrections made in response to the Raw Sequence Listing Error Report, as follows:

The General Information section has been amended to include the application number.

In SEQ ID NO. 1, an inadvertently added amino acid number under stop codon had been deleted, subsequent amino acid numbers have been adjusted and numbers indicating the position of the amino acids have been realigned.

In SEQ ID NO. 5, the numbers indicating the position of the amino acids have been realigned.

These corrections are formal and responsive to the Raw Sequence Listing Error Report and the Notice to File Missing Parts mailed June 19, 2002, and thus no new matter has been added.

* * *

Respectfully submitted,
HELLER EHRMAN WHITE & McAULIFFE LLP

By: ______________________
Stephanie L. Seidman
Registration No. 33,779

Attorney Docket No. 24747-1104US
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10/070489 091702

Rec'd PCT/PTO 17 SEP 2002

PATENT APPLICATION
Attorney Docket No. 24747-1104US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Glare *et al.*
Docket No.:	24747-1104US
Filed:	March 1, 2002
For:	**NUCLEOTIDE SEQUENCES ENCODING AN INSECTICIDAL PROTEIN COMPLEX FROM SERRATIA**

VERIFIED STATEMENT PURSUANT TO 37 § C.F.R. 1.821(f)

I, Megha Bhumralkar, the undersigned, a Patent Scientific Advisor, in the patent practice group of Stephanie Seidman, Esq., declare that I personally prepared the computer-readable copy of the Sequence Listing set forth in above-entitled Application. The computer-readable file is titled 1104SEQ.US2 on the disk provided herewith.

I further declare that the computer-readable form of the SEQUENCE LISTING is identical to the written form of the replacement sequence listing and that the sequence listing does not contain matter that goes beyond the scope of the disclosure contained in the above-identified Application.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Dated at San Diego, California this 1st day of August, 2002.

MBhumralkar

Megha Bhumralkar
Patent Scientific Advisor to
Stephanie L. Seidman
Registration No. 33,779
Attorney for Applicant

10/070489
JC13 Rec'd PCT/PTO 01 MAR 2002

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Glare *et al.*

National Stage of International Appln. No.: PCT/NZ00/00174
Filed: 04 September 2000

Filed: herewith

For: NUCLEOTIDE SEQUENCES ENCODING AN INSECTICIDAL PROTEIN COMPLEX FROM SERRATIA

Group Art Unit: unassigned

Examiner: unassigned

ATTACHMENT TO THE PRELIMINARY AMENDMENT
MARKED UP PARAGRAPHS AND CLAIMS (37 CFR §1.121)

IN THE CLAIMS

Please amend claims 8, 15 and 34 as follows:

8. (Amended) A purified and isolated nucleic acid molecule [as claimed in any one] of [claims] claim 4[through 6].

15. (Amended) A polypeptide resulting form the transformation or transfection of a host cell with a recombinant expression vector [as claimed in any one] of claim [claims] 12 [through 14].

34. (Amended) An insecticidal composition [as claimed in] of claim 32,[or 33] wherein the composition further comprises additional pesticides[, including compounds known to possess herbicidal,fungicidal, insecticidal or nematicidal activity].

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Glare *et al.*

National Stage of International Appln. No.: PCT/NZ00/00174
Filed: 04 September 2000

Filed: herewith

For: NUCLEOTIDE SEQUENCES ENCODING AN INSECTICIDAL PROTEIN COMPLEX FROM SERRATIA

Group Art Unit: unassigned

Examiner: unassigned

CERTIFICATE OF MAILING BY "EXPRESS MAIL"

"Express Mail" Mailing Label Number EL870637462US
Date of deposit March 1, 2002

I hereby certify that this paper and the attached papers are being deposited with the United States Postal "Express Mail Post Office to Addressee" Service under 37 C.F.R. §1.10 on the date indicated above and addressed to: Commissioner of Patents and Trademarks, P.O. Box 2327, BOX PCT, Arlington, VA 22202.

Alicia Bradbury
Alicia Bradbury

PRELIMINARY AMENDMENT

BOX PCT
Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

Preliminary to the examination of the above-captioned application, please amend the application as follows:

IN THE CLAIMS:

Please add claims 42-48 as follows:

42. (New) A purified and isolated nucleic acid molecule of claim 5.

43. (New) A purified and isolated nucleic acid molecule of claim 6.

44. (New) An insecticidal composition of claim 33, wherein the composition further comprises additional pesticides.

45. (New) The insecticidal composition of claim 34, wherein an additional pesticide comprises a compound that has herbicidal, fungicidal, insecticidal or nematicidal activity.

46. (New) The insecticidal composition of claim 44, wherein an additional pesticide comprises a compound that has herbicidal, fungicidal, insecticidal or nematicidal activity.

47. (New) A polypeptide resulting form the transformation or transfection of a host cell with a recombinant expression vector of claim 13.

48. (New) A polypeptide resulting form the transformation or transfection of a host cell with a recombinant expression vector of claim 14.

Please replace claims 8, 15 and 34 with amended claims 8, 15 and 34 as follows:

8. (Amended) A purified and isolated nucleic acid molecule of claim 4.

15. (Amended) A polypeptide resulting form the transformation or transfection of a host cell with a recombinant expression vector of claim 12.

34. (Amended) An insecticidal composition of claim 32, wherein the composition further comprises additional pesticides.

IN THE SPECIFICATION

Between the Title and "Technical Field", on page 1 of the specification, insert:

—This application is the National Stage of International Application. No. PCT/NZ00/00174, filed 04 September 2000. Benefit of priority under 35 U.S.C. §365(b) to New Zealand application no. 337610, filed 02 September 1999 is claimed herein.—

REMARKS

Any fees that may be due in connection with filing this paper or this application during its pendency may be charged to Deposit Account No. 50-1213.

Claims 1-48 are presently pending. The claims are amended and new claims 42-48 added herein to delete multiple dependencies. The specification is amended to reflect the priority claim. Therefore, no new matter has been added nor have any amendments that alter the scope of the claims been introduced.

It is respectfully requested that any references of record in the International stage of prosecution of this application be made of record in this application.

Included as an attachment is a marked-up version of the amended claims pursuant to 37 C.F.R. §1.121.

* * *

In view of the above amendments and remarks, reconsideration and allowance of the application are respectfully requested.

Respectfully submitted,
HELLER EHRMAN WHITE & McAULIFFE LLP

By: ______________________________
Stephanie Seidman
Registration No. 33, 779

Attorney Docket No. 24747-1104US

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10/070489
PCT/NZ00/00174
Rec'd PCT/PTO 01 MAR 2002

NUCLEOTIDE SEQUENCES

TECHNICAL FIELD

The present invention concerns novel nucleotide sequences encoding insecticidal proteins from the Enterobacteriaceae, *Serratia entomophila* and *Serratia proteamaculans,* and the use of said nucleotide sequences and insecticidal proteins.

BACKGROUND ART

Some *Serratia entomophila* and *Serratia proteamaculans* strains in New Zealand are known to cause a disease in the major scarab pest, *Costelytra zealandica* (New Zealand grass grub). The disease was first discovered and described by Trought and Jackson (1982) and was later named amber disease after the distinctive colour of affected insects (Stucki et al. 1984). One species capable of causing the disease, *Serratia entomophila,* was developed into a commercially-available product ("Invade") in 1989.

The disease is highly host specific, only know to infect a single indigenous species of New Zealand scarab larva. The disease appears unique among insects and results not from rapid invasion of the haemocoel, but from a slow colonisation of the gut. The disease has a distinct phenotypic progression, with infected hosts ceasing feeding within 2-5 days of ingesting pathogenic cells. The normally black gut clears around this time (Jackson et al. 1993) and the levels of the major gut digestive enzymes (trypsin and so forth) decreases sharply (Jackson, 1995). The clearance of the gut results in a characteristic amber colour of the infected hosts. The larvae may remain in this state for a prolonged period (1-3 months) before bacteria eventually invade the haemocoel, causing rapid death.

The finding of a plasmid that apparently encoded the disease was reported in Glare et al. (1993) by showing a correlation between pADAP presence and disease occurrence in

bacterial strains. This was further confirmed by Glare et al. (1996) who showed that transfer of the plasmid from pathogenic to non-pathogenic strains resulted in a change to pathogenic.

Grkovic et al. (1995) showed that disruption of the plasmid by transposon insertion could alter pathogenicity without fully defining the area containing the gene cassette. By marker exchange, they showed that a 10.5kb *Hin*dIII (pGLA20) construct from pADAP encoded some functions of amber disease. However, the clone did not contain all disease encoding plasmid-borne regions.

Another region involved in amber disease encoding was located by Nunez-Valdez and Mahanty (1996). They located a locus, *amb2*, by transposon mutagensis and searching a cosmid genomic library. This region was chromosomally located and was involved in antifeeding in the larvae of *Costelytra zealandica*. However, the current applicant's research has demonstrated that the *amb2* region is located on pADAP remote from the virulence gene and is probably regulatory in function.

Insecticidal toxins which share some protein homology to the *Serratia* insecticidal proteins of the present invention have been recently discovered (PCT/US96/18803; PCT/US97/07657) by a group at Wisconsin University (Blackburn et al. 1998; Bowen et al. 1998; Bowen and Ensign 1998). These insecticidal toxins are produced from a gene region in *Photorhabdus luminescens* which resembles the *Serratia* virulence region in the clustering of the genes and at the protein level, but has very little DNA homology with the *Serratia* genes. They have shown high molecular weight proteins from *Photorhabdus luminescens* are insecticidal to a number of insects from different orders. The lack of DNA homology over the majority of the region, as opposed to protein homology, between the *Serratia* genes and *Photorhabdus* genes suggests that these proteins have evolved as a result of convergent evolution leading to the formation of a distinct protein family with a

common function.

The present applicant has now found that three regions of the pADAP plasmid are required for full insecticidal function. Sequence analysis of these three regions has shown that the present applicant has isolated and identified a novel toxin from *Serratia* species that belongs to a new family of insecticidal toxins. It is broadly to this toxin that the present invention is directed.

DISCLOSURE OF INVENTION

According to a first aspect of the present invention, there is provided an isolated nucleic acid molecule comprising a nucleotide sequence of SEQ ID NO: 1 which encodes an insecticidal protein complex, or a functional fragment, neutral mutation, or homolog thereof which have at least 75% nucleic acid homology to SEQ ID NO: 1 and are capable of hybridising with said nucleic acid molecule under stringent hybridisation conditions.

The invention also provides an isolated nucleic acid molecule comprising the nucleotide sequence 1995-18937 of SEQ ID NO: 1 which encodes an insecticidal protein complex, or a functional fragment, neutral mutation, or homolog thereof capable of hybridising with said nucleic acid molecule under standard hybridisation conditions.

The invention also provides an isolated nucleic acid molecule comprising one or more of the nucleotide sequences 2411-9547, 9589-13883 or 14546-17467 of SEQ ID NO: 1 which encode insecticidal proteins, or a functional fragment, neutral mutation, or homolog thereof capable of hybridising with said nucleic acid molecule under standard hybridisation conditions.

Preferably the nucleic acid molecule comprises all of nucleotide sequences 2411-9547, 9598-13884 and 14546-17467 of SEQ ID NO: 1.

The invention further relates to an isolated nucleic acid molecule comprising a sequence of SEQ ID NO: 1, nucleotides 1955-18937 of SEQ ID NO: 1 or one or more of nucleotides 2411-9547, 9598-13884 or 14546-17467 of SEQ ID NO: 1, operably linked to at least one further nucleotide sequence which encode an insecticidal protein. For example, the at least one further nucleotide sequence may be the nucleotide sequence which codes for the *Bacillus* delta endo toxins, vegatative insecticidal proteins (vips), cholesterol oxidases, *Clostridium bifermentens* mosquitocidal toxins and/or *Photorhabadus luminescens* toxins and so forth.

The nucleic acid molecule may comprise DNA, cDNA or RNA.

Preferably said fragment, neutral mutation or homolog thereof is capable of hybridising to said nucleic acid molecule under stringent hybridisation conditions.

The invention further relates to nucleic acid molecules which hybridise to the nucleotide sequence of SEQ ID NO: 1, or nucleotides 1955-18937, 2411-9547, 9598-13884 or 14546-17467 of SEQ ID NO: 1 if there is at least 75% or greater identity between the sequences.

The nucleic acid molecule may be isolated from *Serratia entomophila* or *Serratia proteamaculans* strains.

Also provided by the present invention are recombinant expression vectors containing the nucleic acid molecule of the invention and hosts transformed with the vector of the invention capable of expressing a polypeptide of the invention.

The vector may be selected from any suitable natural or artificial plasmid/vector. For example, pUC 19 (Yannish-Perron et al. 1995), pProEX HT (GibcoBRL, Gaithersburg, MD, USA), pBR322 (Bolivar et al. 1977), pACYC184 (Chang et al. 1978), pLAFR3 (Staskowicz et al. 1987), and so forth.

In a further aspect, the invention provides a method of producing a polypeptide of the invention comprising the steps of:

(a) culturing a host cell which has been transformed or transfected with a vector as defined above to express the encoded polypeptide or peptide; and

(b) recovering the expressed polypeptide or peptide.

An additional aspect of the present invention provides a ligand that binds to a polypeptide of the invention. Most usually, the ligand is an antibody or antibody binding fragment. Such ligands also form a part of this invention.

According to a further aspect of the present invention there are provided probes and primers comprising a fragment of the nucleic acid molecule of the invention capable of hybridising under stringent conditions to a native insecticidal gene sequence. Such probes and primers are useful, for example, in studying the structure and function of this novel gene and for obtaining homologs of the gene from bacteria other than *Serratia* sp.

According to a still further aspect of the present invention there is provided a polypeptide having insecticidal activity encoded by the nucleic acid molecule of the invention, or a functional fragment, neutral mutation or homolog thereof.

The polypeptide may comprise the amino acid sequence of SEQ ID NO: 1 or a functional fragment, neutral mutation or homolog thereof.

The polypeptide may comprise amino acids 32-5118 of SEQ ID NO: 1.

The polypeptide may comprise at least one amino acid sequence of SEQ ID NO: 2; SEQ ID NO: 3; SEQ ID NO: 4; SEQ ID NO: 5 or SEQ ID NO: 6.

Preferably the polypeptide comprises amino acid sequence SEQ ID NO: 4; SEQ ID NO: 5

and SEQ ID NO: 6.

More preferably the polypeptide comprises all of SEQ ID NOs: 2-6.

Conveniently, the polypeptide of the invention is obtained by expression of a DNA sequence coding therefore in a host cell or organism.

The polypeptide may comprise the amino acid sequence of SEQ ID NO: 1 linked to at least one further amino acid sequence encoding an insecticidal protein. For example, the at least one further amino acid sequence may be the amino acid sequence which codes for *Bacillus* delta endo toxins, vegatative insecticidal proteins (vips), cholesterol oxidases, *Clostridium bifermentens* mosquitocidal toxins and/or *Photorhabadus luminescents* toxins etc.

The invention further relates to polypeptides comprising at least 50%, preferably 60%, more preferably 70% and most preferably 90-95% or greater identity to SEQ ID NO: 1.

The polypeptide may be produced by expression of a vector comprising the nucleic acid molecule of the invention or a functional fragment, neutral mutation or homolog thereof, in a suitable host cell.

According to a further aspect, there is provided an insecticidal composition comprising at least the polypeptide of the invention and an agriculturally acceptable carrier such as would be known to a person skilled in the art. More than one polypeptide of the invention can of course, be included in the composition. In addition, the composition may comprise one or more additional pesticides, for example, compounds known to possess herbicidal, fungicidal, insecticidal or nematicidal activity.

The composition may further comprise other known insecticidally active agents, such as *Bacillus* delta endo toxins, vegatative insecticidal proteins (vips), cholesterol oxidases, *Clostridium bifermentens* mosquitocidal toxins and/or *Photorhabadus luminescents* toxins

and so forth.

According to a further aspect, there is provided a method of combating pests, especially insects at a locus or host for the pest infested with or liable to be infested therewith, said method comprising applying to a locus, host and/or the pest, an effective amount of the polypeptide of the invention that has functional insecticidal activity against said pest.

According to a further aspect the invention provides a method of inducing amber disease or like condition in insects comprising delivery to an insect an effective amount of the polypeptide of the invention that has functional insecticidal activity against said insect.

The insect may be selected from the order comprising Coleoptera (such as the black beetle, *Heteronychus arator* (F.), or the black vine weevil, *Otiorhynchus sulcatus* (F.)); Dictyoptera (eg. The German cockroach, *Blattella germanica* (L.), or the subterranean termite *Coptotermes* spp,); Diptera (eg. the housefly *Musca domestica* L. or the blowfly *Lucillia cuprina* (Wiedermann); Orthoptera (eg. The black field cricket *Telleogryllus commodus* (Walker) or the migratory locust *Locusta migratoria* L.); Hymenoptera (eg. The German wasp, *Vespula germanica* F.)); Hemiptera (such as the green vegetable bug *Nezara viridula* (L.) or the green peach aphid *Myzus persicae* (Sulzer)) the Lepidoptera (eg. the tomato fruitworm, *Helicoverpa armigera* (Walker), or the codling moth, *Laspeyresia pomonella* (L.)).

The insecticidal polypeptide may be delivered to the insect orally either as a solid bait matrix, as a sprayable insecticide sprayed onto a substrate upon which the insect feeds, applied directly to the soil subsurface or as a drench or is expressed in an transgenic plant, bacterium, virus or fungus upon which the insect feeds, or by any other suitable method which would be obvious to a person skilled in the art.

According to a further aspect, the invention provides a transgenic plant, bacterium virus or

fungus, incorporating in its genome, a nucleic acid molecule of the invention providing the plant, bacterium virus or fungus with an ability to express an effective amount of an insecticidal polypeptide.

DEFINITIONS AND METHODS

The following definitions and methods are provided to better define the present invention and to guide those of ordinary skill in the art in the practice of the present invention.

Definitions of common terms in molecular biology may also be found in Lewin, *Genes V*, Oxford University Press: New York, 1994.

The term "native" refers to a naturally-occurring nucleic acid or polypeptide, including, wild-type sequence and alleles thereof.

A "homolog" has at least one of the biological activities of the nucleic acid or polypeptide of the invention and comprises at least 50-70% identical amino acid or nucleic acid sequence thereto, preferably 75-85% and most preferably 90-95% identical amino acid or nucleic acid sequence thereto.

The term "neutral mutation" means a mutation, (that is - a change in the nucleotide or polypeptide sequence such as by deletion, substitution, inversion or insertion, any of which have no effect on the function of the encoded protein).

As indicated above, also possible are variants of the polypeptide or peptide that differ from the native amino acid sequence by insertion, substitution or deletion of one or more amino acids. Where such a variant is desired, the nucleotide sequence of the native DNA is altered appropriately. This alteration can be made through elective synthesis of the DNA, or by modification of the native DNA by, for example, site specific or cassette mutagenesis. Preferably, where portions of cDNA or genomic DNA require sequence modifications, site-

specific primer directed mutagenesis is employed using techniques standard in the art.

In a further aspect, the present invention consists in replicable transfer vector suitable for use in preparing a polypeptide of the invention. These vectors may be constructed according to techniques well known in the art, or may be selected from cloning vecotrs available in the art.

The cloning vector may be selected according to the host or host cell to be used. Useful vectors will generally have the following characteristics:

(a) the ability to self-replicate;

(b) the possession of a single target for any particular restriction endonuclease; and

(c) desirably, carry genes for a readily selectable marker such as antibiotic resistance.

Two major types of vector possessing these characteristics are plasmids and bacterial viruses (bacteriophages or phages). Presently preferred vectors include plasmids pMOS-Blue, pGem-T and pUC8.

The nucleic acids of the present invention can be free in solution, or attached by conventional means to a solid support, or present in an expression vector or any other type of plasmid.

The term "isolated" means substantially separated or purified away from contaminating sequences in the cell or organism in which the nucleic acid naturally occurs and includes nucleic acids purified by standard purification techniques as well as nucleic acids prepared by recombinant technology and those chemically synthesised.

The terms "DNA construct" means a construct incorporating the nucleic acid molecule of the present invention, or a fractional fragment, neutral mutation or homolog thereof in a

position whereby the protein coding sequence is under the control of an operably linked promoter capable of expression in a plant cell. Such promoters are well known in the art.

A fragment of a nucleic acid molecule according to the present invention is a portion of the nucleic acid that is less than full length and comprises at least a minimum length capable of hybridising specifically with a nucleic acid molecule according to the present invention (or a sequence complementary thereto) under stringent conditions as defined below. A fragment according to the present invention has at least one of the biological activities of the nucleic acid or polypeptide of the present invention.

Nucleic acid probes and primers can be prepared based on nucleic acids according to the present invention (for example, the sequence of SEQ ID NO: 1). A "probe" comprises an isolated nucleic acid attached to a detectable label or reporter molecule well known in the art. Typical labels include radioactive isotopes, ligands, chemiluminescent agents, and enzymes.

"Primers" are short nucleic acids, preferably DNA oligonucleotides 15 nucleotides or more in length, which are annealed to a complementary target DNA strand by nucleic acid hybridisation to form a hybrid between the primer and the target DNA strand, then extended along the target DNA strand by a polymerase, preferably a DNA polymerase. Primer pairs can be used for amplification of a nucleic acid sequence, (for example, by the polymerase chain reaction (PCR) or other nucleic acid amplification methods well known in the art). PCT-primer pairs can be derived from the sequence of a nucleic acid according to the present invention, (for example, by using computer programs intended for that purpose such as Primer (Version 0.5© 1991, Whitehead Institute for Biomedical Research, Cambridge, MA)).

Methods for preparing and using probes and primers are described, for example, in Sambrook et al. *Molecular Cloning: A Laboratory Manual*, 2nd ed, vol. 1-3, ed Sambrook

et al. Cold Spring Harbour Laboratory Press, Cold Spring Harbour, NY, 1989.

Probes or primers can be free in solution or covalently or noncovalently attached to a solid support by standard means.

The term "operably linked" means a first nucleic acid sequence linked to a second nucleic acid sequence when the first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence. For instance, a promoter is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence. Generally, operably linked DNA sequences are contiguous and, where necessary to join two protein coding regions, in reading frame.

The DNA molecules of the invention may be expressed by placing them in operable linkage with suitable control sequences in a replicable expression vector. Control sequences may include origins of replication, a promoter, enhancer and transcriptional terminator sequences, amongst others. The selection of the control sequence to be included in the expression vector is dependent on the type of host or host cell intended to be used for expressing the DNA.

A "recombinant" nucleic acid is one that has a sequence that is not naturally occurring or has a sequence that is made by an artificial combination of two otherwise separated segments of sequence. This artificial combination is often accomplished by chemical synthesis or, more commonly, by the artificial manipulation of isolated segments of nucleic acids (for example, by genetic engineering techniques).

Techniques for nucleic acid manipulation are described generally in, for example, Sambrook et al. (1989).

Large amounts of a nucleic acid according to the present invention can be produced by recombinant means well known in the art or by chemical synthesis.

Natural or synthetic nucleic acids according to the present invention can be incorporated into recombinant nucleic acid constructs, typically DNA constructs, capable of introduction into and replication in a host cell. Usually the DNA constructs will be suitable for replication in a unicellular host, such as *E. coli* or other commonly used bacteria, but can also be introduced into yeast, mammalian, plant or other eukaryotic cells.

Preferably, such a nucleic acid construct is a vector comprising a replication system recognised by the host. For the practice of the present invention, well known compositions and techniques for preparing and using vectors, host cells, introduction of vectors into host cells and so forth., are employed, as discussed, *inter alia,* in Sambrook et al (1989).

A cell, tissue, organ, or organism into which has been introduced a foreign nucleic acid, such as a recombinant vector, is considered "transformed" or "transgenic". The DNA construct comprising a DNA sequence according to the present invention that is present in a transgenic host cell, particularly a transgenic plant, is referred to as a "transgene". The term "transgenic" or "transformed" when referring to a cell or organism, also includes;

(1) progeny of the cell or organism, and

(2) plants produced from a breeding program employing such a "transgenic" plant as a parent in a cross and exhibiting an altered phenotype resulting from the presence of the recombinant DNA construct.

Generally, procaryotic, yeast, insect, or mammalian cells are useful hosts. Also included within the term hosts are plasmid vectors. Suitable procaryotic hosts include *E. coli, Bacillus* species and various species of *Pseudomonas*. Commonly used promoters such as β-lactamase (penicillinase) and lactose (lac) promoter systems are all well known in the art. Any available promoter system compatible with the host of choice can be used. Vectors used in yeast are also available and well known. A suitable example is the 2 micron origin

of replication plasmid.

Similarly, vectors for use in mammalian cells are also well known. Such vectors include well known derivatives of SV-40, adenovirus, retrovirus-derived DNA sequences, *Herpes simplex* virus, and vectors derived from a combination of plasmid and phage DNA.

Further eucaryotic expression vectors are known in the art (for example in P.J. Southern and P. Berg, *J. Mol. Appl. Genet. 1* 327-341 (1982); S. Subramani et al., *Mol. Cell. Biol. 1*, 854-864 (1981); R.J. Kaufmann and P.A. Sharp, "*Amplification and Expression of Sequences Cotransfected with a Modular Dihydrofolate Reducase Complementary DNA Gene*, *J. Mol. Biol. 159*, 601-621 (1982); R.J. Kaufmann and P.A. Sharp, *Mol. Cell. Biol. 159*, 601-664 (1982); S.I. Scahill et al., "*Expressions and Characterisation of the Product of a Human Immune Interferon DNA Gene in Chinese Hamster Ovary Cells*," *Proc. Natl. Acad. Sci. USA. 80*, 4654-4659 (1983); G. Urlaub and L.A. Chasin, *Proc. Natl. Acad. Sci. USA. 77*, 4216-4220, (1980).

The expression vectors useful in the present invention contain at least one expression control sequence that is operatively linked to the DNA sequence or fragment to be expressed. The control sequence is inserted in the vector in order to control and to regulate the expression of the cloned DNA sequence. Examples of useful expression control sequences are the <u>lac</u> system, the <u>trp</u> system, the <u>tac</u> system, the <u>trc</u> system, major operator and promoter regions of phage lambda, the glycolytic promoters of yeast acid phosphatase, (for example, Pho5), the promoters of the yeast alpha-mating factors, and promoters derived from polyoma, adenovirus, retrovirus, and simian virus (for example, the early and late promoters of SV-40), and other sequences known to control the expression of genes of prokaryotic and eucaryotic cells and their viruses or combinations thereof.

In the construction of a vector it is also an advantage to be able to distinguish the vector incorporating the foreign DNA from unmodified vectors by a convenient and rapid assay.

Reporter systems useful in such assays include reported genes, and other detectable labels which produce measurable colour changes, antibiotic resistance and the like. In one preferred vector, the β-galactosidase reporter gene is used, which gene is detectable by clones exhibiting a blue phenotype on X-gal plates. This facilitates selection. In one embodiment, the β-galactosidase gene may be replaced by a polyhedrin-encoding gene; which gene is detectable by clones exhibiting a white phenotype when stained with X-gal.

This blue-white colour selection can serve as a useful marker for detecting recombinant vectors.

Once selected, the vectors may be isolated from the culture using routine procedures such as freeze-thaw extraction followed by purification.

For expression, vectors containing the DNA of the invention to be expressed and control signals are inserted or transformed into a host or host cell. Some useful expression host cells include well-known prokaryotic and eucaryotic cells. Some suitable prokaryotic hosts include, for example, *E. coli*, such as *E. coli*, S G-936, *E. coli* HB 101, *E. coli* W3110, *E. coli* X1776, *E. coli*, X2282, *E. coli* DHT and *E. coli* MR01, *Pseudomonas*, *Bacillus*, such as *Bacillus subtilis* and *Streptomyces*. Suitable eucaryotic cells include yeast and other fungi, insect, animal cells, such as COS cells and CHO cells, human cells and plant cells in tissue culture.

Depending on the host used, transformation is performed according to standard techniques appropriate to such cells. For prokaryotes or other cells that contain substantial cell walls, the calcium treatment process (Cohen, S N *Proceedings, National Academy of Science, USA* 69 2110 (1972)) may be employed. For mammalian cells without such cell walls the calcium phosphate precipitation method of Graeme and Van Der Eb, *Virology* 52:546 (1978) is preferred. Transformations into plants may be carried out using *Agrobacterium tumefaciens* (Shaw et al., Gene 23:315 (1983)) or into yeast according to the method of Van

Solingen et al. *J. Bact.* 130:946 (1977) and Hsiao et al. *Proceedings, National Academy of Science,* 76:3829 (1979).

Upon transformation of the selected host with an appropriate vector the polypeptide, or peptide encoded can be produced, often in the form of fusion protein, by culturing the host cells. The polypeptide, or peptide, of the invention may be detected by rapid assays as indicated above. The polypeptide, or peptide, is then recovered and purified as necessary. Recovery and purification can be achieved using any of those procedures known in the art, for example by absorption onto the elution from an anion exchange resin. This method of producing a polypeptide, or peptide, of the invention constitutes a further aspect of the present invention.

Host cells transformed with the vectors of the invention also form a further aspect of the present invention.

Methods for chemical synthesis of nucleic acids are well known and can be performed, for example, on commercial automated oligonucleotide synthesisers.

The term "stringent conditions" is functionally defined with regard to the hybridisation of a nucleic acid probe to a target nucleic acid (for example, to a particular nucleic acid sequence of interest) by the hybridisation procedure discussed in Sambrook et al. (1989) at 9.52-9.55 and 9.56-9.58.

Regarding the amplification of a target nucleic acid sequence (for example,. by PCR) using a particular amplification primer pair, stringent conditions are conditions that permit the primer pair to hybridise only to the target nucleic acid sequence to which a primer having the corresponding wild type sequence (or its complement) would bind.

Nucleic acid hybridisation is affected by such conditions as salt concentration, temperature, or organic solvents, in addition to the base composition, length of the complementary

strands, and the number of nucleotide base mismatches between the hybridising nucleic acids, as will be readily appreciated by those skilled in the art.

When referring to a probe or primer, the term "specific for (a target sequence)" indicates that the probe or primer hybridises under stringent conditions only to the target sequence in a given sample comprising the target sequence.

The term "protein (or polypeptide)" refers to a protein encoded by the nucleic acid molecule of the invention including fragments, mutations and homologs having the same biological activity (for example, insecticidal activity). The polypeptide of the invention can be isolated from a natural source, produced by the expression of a recombinant nucleic acid molecule or be chemically synthesised.

Peptides having substantial sequence identity to the above-mentioned peptides can also be employed in preferred embodiments. Here, "substantial sequence identity" means that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 80% sequence identity, preferably at least 90% sequence identity, more preferably at least 95% sequence identity or more. Preferably, residue positions that are not identical differ by conservative amino acid substitutions. For example, the substitution of amino acids having similar chemical properties such as charge or polarity are not likely to effect the properties of a protein. Examples include glutamine for asparagine, or glutamic acid for aspartic acid.

BRIEF DESCRIPTION OF DRAWINGS

The invention will be further defined by reference to the specification and the following examples and figures herein.

Figure 1 shows restriction maps of clones used to isolate the pathogenic region and maps of the two pathogenic variants pMH32 and pMH41, in accordance

with a preferred embodiment of the present invention; and

Figure 2 shows deletion derivatives used in the study, restriction maps of the mutated constructs and recombinants, the phenotype of each mutation, the schematic diagram of the sequenced region, and a nucleotide sequence in accordance with a preferred embodiment of the present invention; and

Figure 3 shows hydrophobicity plots of SepC and its closest homologue TccC, in accordance with a preferred embodiment of the present invention; and

Figure 4 shows the comparison of protein sequences of the SepA and *P. luminescens* toxins, TcdA, TcaB and TccB Putative RGD motif is boxed, plus the site of proteolytic cleavage is illustrated, in accordance with a preferred embodiment of the present invention; and

Figure 5 shows the comparison of protein sequences of the SepC and *P. luminescens* toxin TccC, in accordance with a preferred embodiment of the present invention; and

Figure 6 shows the plasmid pADAP, in accordance with a preferred embodiment of the present invention.

BEST MODES FOR CARRYING OUT THE INVENTION

The invention will be further defined by reference to the specification and the following examples and figures herein in the ensuing description by way of example only where:

Figure 1 shows restriction maps of clones used to isolate the pathogenic region and maps of the two pathogenic variants pMH32 and pMH41, where:

(A) Is the pADAP *Hind*III clone pGLA-20 showing locations of the pGLA-20 mutations –

10, -13, and 35, which when recombined back into pADAP and bioassayed against grass grub, result in either a pathogenic phenotype, shown by full flag, or a healthy but non-feeding phenotype indicated by half filled flag. Map of pBG35 showing relative position of pGLA-20-35 mutation and the location of the 2.2kb *Eco*Ri used as a probe to screen the pADAP *Bam*HI library; and

(B) Illustrated restriction enzyme maps of the pathogenic clones pMH32 and pMH41, area of deletion is indicated by Δ.

pBR322 vector DNA;

pLAFR3 vector DNA.

Restriction enzymes are abbreviated as follows: B, *Bam*HI, Bg, *Bgl*II; E, *Eco*RI: H, *Hind*III; and X, *Xba*I.

Figure 2 shows:

(A) Which are Mini-Tn*10* pACYC184 based deletion derivatives used in the study.

is the pACYC184 vector,

Δ indicates deletion + pathogenic,

- loss of pathogenicity; and

(B) Illustrates restriction maps of the mutated constructs pBM32 and the pADK recombinants; and

(C) Where the phenotype of each mutant is indicated by flags.

Blocked flags indicates mutations that did not affect the disease process.

Open flags indicate mutations that abolish disease symptoms.

Half-filled flags denote mutations that abolish visual disease symptoms but are unable to feed.

* indicates pADK mutations obtained by Grkovic et al. (1995).

Restriction enzymes are abbreviated as follows: B, *Bam*HI, Bg, *Bgl*II; E, *Eco*RI; H, *Hind*III; and X, *Xbal.*

(D) Is a schematic diagram of the sequenced region, where:

Denotes sequenced region.

Arrows indicate ORFs and their direction

region homologous to spvB ... location of repeat.

(E) Is a nucleotide sequence of the 5 times 12bp repeat and the palindrome.

Restriction enzymes are abbreviated as follows: B, *Bam*HI, Bg, *Bgl*II; E, *Eco*RI; H, *Hind*III; and X, *Xbal.*

In Figure 3 hydrophobicity plots of SepC and its closest homologue TccC are shown. The scale is disproportional to size and has a scanning window of 17 amino-acid residues.

Figure 4 shows the comparison of protein sequences of the SepA and *P. luminescens* toxins, TcdA, TcaB and TccB. Putative RGD motif is boxed. The site of proteolytic cleavage is reported by Bowen et al. (1998) (Residue 1933 of TcdA) is indicated by an arrow.

Figure 5 shows the comparison of protein sequences of the SepC and *P. luminescens* toxin TccC; and Figure 6 shows the plasmid pADAP.

PROTOCOL

Bacterial isolates and methods of culture

Table 1 lists bacterial isolates and plasmids used in the present invention. Bacteria were grown in LB broth or on LB agar (Sambrook et al. 1989), at 37° for *Escherichia coli* and 30°C for *S. entomophila*. Antibiotic concentrations used (µg/ml) for *Serratia* were kanamycin 100, chloramphenicol 90, tetracycline 30 and for *E. coli* strains were kanamycin 50, chloramphenicol 30, tetracycline 15, and ampicillin 100.

DNA isolation and manipulations

pADAP DNA was isolated from a 50ml overnight culture of bacteria using QIAGEN® plasmid maxi kit (Qiagen, Hilden, Germany), as per the manufacturer's instructions. Standard DNA techniques were carried out as described by Sambrook et al. (1989). Radioactive probes were made using the Amersham Megaprime DNA labeling system (Amersham, Buckinghamshire, UK). Southern and colony hybridisations were performed as outlined in Sambrook et al. (1989). The plasmid pADAP is shown in Figure 6.

pADAP *Bam*HI library was constructed using a Sigma 'Gigapack®IIIXL packaging extract, as specified by the manufacturer (Stratagene, California, USA).

Introduction of plasmid DNA into *E. coli* and *S. entomophilia*

pLAFR3 based derivatives were introduced into *S. entomophilia* by tripartite matings on solid media as described previously (Finnegan & Sheratt, 1982) using the pRK2013 helper plasmid (Figorski & Helanski, 1979). pACYC184 and pBR322 based plasmids were electroporated into *E. coli* and *S. entomophilia* strains, using a Biorad Gene Pulser (2µF, 2.5KV, and 200 abns) (Dower et al. 1988).

Mutagenesis

Transposon insertions were generated in recombinant plasmids using the mini-*Tn10* derivative 103 (kanamycin resistant) as described by Kleckner et al. (1991). Insertions were recombined into pADAP by transforming A1MO2 (refer to Table 1) with the described construct. After growth in non-selective media, bacteria were screened for resistance to kanamycin and loss of the pLAFR3 tetracycline resistance marker.

Bioassay against *Costelytra zealandica* larvae

Infection of *C. zealandica* larvae was determined by a standard bioassay where the healthy larvae, collected from the field, were individually fed squares of carrot which had been rolled in colonies of bacteria grown overnight on solid media (resulting in approximately 10^5 cells/carrot square). Twelve, second or third instar larvae were used for each treatment. Inoculated larvae were maintained at 15°C, in ice-cube trays. Larvae were left feeding on treated carrot for 3-4 days, then transferred to fresh trays and provided with untreated carrot for 10-14 days. The occurrence of gut clearance and loss of feeding was recorded every 3-4 days. Strains were considered disease-causing if greater than 70% of larvae showed disease symptoms by day 14. Known pathogenic and non pathogenic controls were included in all bioassays. Typically cessation of feeding occurs within 2-3 days while clearance of the larvae gut may take 4-6 days.

Recovery of bacteria from larvae

To isolate bacteria from inoculated grubs, larvae were surface sterilised by submerging in 70% methanol for 30 seconds. The larvae were then shaken in sterile DH_20, removed and individually macerated in a 1.5ml microcentrifuge tube. The macerate was serial diluted and plated on LB media containing antibiotics selective for the host *S. entomophilia* strain. To assess the stability of the bioassayed plasmid, colonies were patched onto a plate

containing antibiotics either selective for the recombinant plasmid or the *S. entomophilia* strain. Identity of plasmids in the recovered strain was checked by restriction enzyme profile.

Nucleotide Sequencing

A 9-kb *Bam*HI –*Eco*RI fragment derived from the pBM32-8 mutation (Fig 2b) and the 8kb *Hind*III fragment of pBM32 were separately cloned into the appropriate site of the deletion factory plasmid pDELTA1. Deletions were generated using the Delection factory™ system (GIBCO BRL, MD, USA), as outlined in the manufacturers instructions. To identify the precise location of mini-*Tn10* mutations, the peripheral mini-*Tn10 Bam*HI sites were used in conjunction with the *Bam*HI sites of the pathogenic region to subclone the mini-*Tn10* flanking regions into either pACYC184 or pUC19. Sequences were generated using the mini-*Tn10* specific primer 5'ATGACAAGATGTGTATCCACC3' (Kleckner et al. 1991).

Plasmids for sequencing were prepared by Wizard® (Promega, Madison, USA) or Quantum Prep® (Bio-Rad, California, USA) miniprep kits. Sequences were determined on both strands, by using combinations of subcloned fragments, custom primers and deletion products derived from the deletion factory system (Gibco BRL, Madison, USA). The DNA was sequenced using either ^{33}P dCTP and the Thermosequenase cycle sequencing kit (Amersham, Buckinghamshire, UK), or by automated sequencing using an Applied Biosystem 373A or 377 autosequencer. Sequence data were assembled using SEQMAN (DNASTAR Inc., Madison, USA). ORF's were analysed by Gene Jockey. Databases at the National Center for Biotechnology Information were searched by using BLASTN and BLASTX via the www.ncbi.nlm.gov/BLAST.. Searches for DNA palindromes, repeats and inverted repeats were undertaken using DNAMAN (Lynnon Biosoft, Quebec, Canada). Protein motifs were searched using Blocks *(http://www.blocks.fhcrc.org/)*, ExPASy *(http://www.expasy.ch/)*, and Gene Quiz *(http://columba.ebi.ac.uk:8765/gqsrv/submit)*.

The sequences determined in this study have been deposited in Gene Bank under Accession Number AF1335182.

RESULTS

Cloning the disease encoding region from pADAP

Previously, Grkovic et al. (1995) have shown taht the pADK-13 mutation can be complemented with the pADAP 11 kb *Hind*III fragment (pGLA-20). However, the pADK-10 mutation was unable to be complemented with pGLA-20. In an attempt to isolate the region that may complement the pADK-10 mutation the previously described pGLA-20 derived, pADK-35 null mutation (Grkovic et al. 1995) was used as a selective marker (Fig 1), to select the *Bgl*II fragment encompassing both the pADK-10 and pADK-35 mutations. pADK-35 DNA was isolated and digested with the restriction enzyme *Bgl*II. The resultant digest was ligated into the *Bam*HI site of bBR322 to form the construct pBG35 (containing 12.8kb *Bgl*II – mini-*Tn10* fragment). pBG35 was placed separately in *trans* with pADK-10 and pGLA-20, and the resultant strains bioassayed against grass grub larvae. Results showed that pBG35 was able to complement the pADK-10 mutant, but was unable to induce any symptoms of amber disease when placed in *trans* with pGLA-20, indicating that there must be another region on pADAP needed to induce amber disease.

Restriction enzyme data of pGLA-20 and pBG35 suggested that the entire pathogenic region may reside within one of the large *Bam*HI fragments of pADAP. A cosmid *Bam*HI library of pADAP was made and screened using the 2.2kb *Eco*RI fragment derived from pBG35 (Fig 1) as the probe. Several probe positive clones were isolated; all shared similar restriction enzyme profiles. However, one (designated pMH32) was found to be smaller, measuring only 23kb in size compared with the 33kb of the other clones (eg. pMH41; Fig 1b). The difference between pMH32 and pMH41 was found to be a 10kb deletion at the left most end of pMH32 encompassing the one *Hind*III site (Fig 1). *E. coli* strains

containing pMH32 or pMH41 were bioassays against grass grub larvae and found to induce the full symptoms of amber disease (that is - gut clearance and antifeeding activity). However, about ten days after infection a proportion of grass grubs fed the *E. coli* strains were found to recover from a diseased to a healthy phenotype.

The plasmids pMH32 and pMH41 were subsequently introduced into a *S. entomophilia* strain cured of pADAP (5.6RC) and the strains bioassayed against grass grub larvae. The strains gave the same disease progression as wild type and no larvae recovered, suggesting that the region cloned in pMH32 contained all the pathogenic determinants of pADAP.

Effect of copy number and mini-*Tn10* insertions in pBM32 on disease-causing ability

To facilitate mutagenesis and assess the effect of copy number on the disease process, the 23kb *Bam*HI fragment from pMH32 was cloned into the medium copy plasmid pBR322 to give pBM32. A bioassay comparing the ability of pMH32 and pBM32 to induce amber disease against grass grub was undertaken. Results showed that there were no visual differences in the progression of amber disease between pBM32 and pMH32. The construct pBM32 was mutated with the mini-*Tn10* transposon derivative 103, and insertions mapped (Fig 2b). Bioassays of *E. coli* strains containing plasmids of the resultant mutants, showed that the disease determinants were confined within a central 16.9kb region (nucleotides 1955-18937 of SEQ ID NO: 1).

All strains were non-pathogenic or fully pathogenic, and no partial disease phenotypes such as antifeeding, or gut clearance were noted.

To confirm that no sequences at either end of the cloned fragment influenced the disease process, several deletion plasmids were made (Fig 2a). The large fragments resulting from cleavage of the pBM32 -4, -8, -10, -20, -23, -24 and -35 plasmids with *Bam*HI were cloned into the analogues site of pACYC184. The resultant plasmids were transformed into the

non-pathogenic *S. entomophilia* strain 5.6RKm and assessed for pathogenicity. This analysis confirmed that the central 16.9kb region (Fig 2a) was sufficient to induce the disease.

Effect of mini-*Tn10* insertions in pADAP on disease-causing ability

Grkovic et al. (1995) recombined by marker exchange the pGLA-20 based mutations - 10 and -13 into pADAP (Fig 2a). When bioassayed, *S. entomophilia* strains containing either of these mutant plasmids caused a partial condition including cessation of feeding but not gut clearance or amber colouration. This was in contrast to the complete abolition of disease observed in pADAP-cured *S. entomophilia* strains containing mutant pBM32 plasmids with similar insertions.

To determine the disease phenotype of the pBM32-based insertions in a pADAP background, the pBM32 based insertions were transferred into pADAP. pBM32 -1, -2, -4, -5, -6, -8, -9, -10, -21, -24, -30, -31 and -35 DNA fragments containing the inserted transposon and flanking DNA were cloned as independent fragments into pLAFR3 and the inserts recombined back into pADAP by marker exchange (Fig 2c). The resultant recombinant *S. entomophilia* strains were checked by Southern analysis to confirm that recombination had occurred as expected and no pLAFR3 vector sequences were present (data not shown). Mutations that did not affect the disease process in pBM32 also had no effect when recombined back into pADAP. However, strains with the pADAP mutants that totally abolished the disease process when in the pBM32 clone caused non-feeding but not gut clearance of the grubs (Fig 2b, c). Hence, none of the pADAP recombinant strains completely abolished the disease process. This suggests that, while the 16.9kb fragment contains all genes required for pathogenicity, other genes contributing to the antifeeding effect are present on some other part of pADAP.

Assessment of plasmid stability during the course of the bioassay showed that greater than

90% of the recombinant *Serratia* strains contained the clone of interest.

Nucleotide Sequence Analysis of the pathogenic region

The large *Bam*HI fragment (18937 bp) derived from the pBM32-8 was sequenced on both strands using a combination of constructed detections, plasmid subclones and custom made primers. A total continuous sequence of 18937 bp has been deposited in Gene Bank (Accession Number AF135182). Structural analysis of the DNA sequence using DNAMAN showed that there was a 12-bp sequence repealed five times between positions 683 and 743. The repeat is flanked by an upstream 13 base pair palindrome (669-682-bp), and a degenerate 34-bp downstream palindrome (765-799-bp)(Fig 2d,e).

Translation of the nucleotide sequence revealed nine significant open reading frames (ORF's). These together with their putative ribosomal binding sites and their base composition are listed in Table 2. Eight of the ORF's were oriented in the same direction and the other two in the opposite direction (Fig 2d). Sequence similarity searches showed that the deduced products of seven of these ORF's shared similarity with known proteins (Table 3). Products of three of the ORF's showed similarity to different protein components of insecticidal toxins of *Photorhabadus luminescents* (Bowen et al. 1998).

These ORF's have been designated *sep. (sepA, sepB and sepC)* for *Serratia entomophilia* pathogenicity.

Similarities of deduced amino-acid sequences to proteins in current database

Results of database searches for homologues proteins are listed in Table 4.

With reference to Fig 2d and Table 4, the following protein similarities were identified:

The protein product of *sepA,* had high similarity to the *P. luminescents* insecticidal toxin complex protein TcbA, TcdA, TcaB and TccB. These proteins shared three significant

regions of predicted amino-acid similarity, at the amino-terminal region (SepA amino-acid residues (121-178), a central region (SepA amino-acid residues 960-1083) and, with greatest similarity, at the carboxyl terminus (SepA amino-acid residues 1630-2376) Fig. 4). However, there was little amino acid conservation around the putative proteolytic cleavage site of TcaB, TcbA and TcdA identified by Bowen et al. (1998). SepA also contained a region (residues 1057-1345) with weak similarity to the *Clostridium bifermentans* mosquitocidal toxin cbm71 (Barloy et al., 1996).

SepB and the *P. luminescens* insecticidal toxin complex protein TcaC shared similarity throughout their length, and both SepA and TcaC showed high amino-terminal similarity to the *Salmonella* virulence protein spvB (Gullig et al. 1992) (Fig. 5). The similarity of SepB and TcaC to SprB diminishes after SpvB amino acid residue 356.

SepC showed strong similarity to the amino-terminal of the insecticidal toxin complex protein TccC, up to amino-acid residue 663 of SepC. A number of putative bacterial cell wall proteins also have high similarity to SepC, including the wall associated protein precursor *B. subtilis* (WAPA) and members of the *E. coli Rhs* (recombinant hot spot) elements. Strong similarity of SepC was also observed with hypothetical wall-associated proteins from *Coxiella burnetti* and *Bacillus subtilis* (Table 4).

The translated sequences of ORF1 and ORF2 showed no similarity to sequences in the current databases. ORF3 shared significant similarity to the morphogenesis protein of the *Bacillus subtilis* bacteriophage B103, a member of bacteriophage muramidase-type lysis proteins (Pecenkova et al. 1996). However, relative to size, the gp19 protein of *S. typhimurium* phage ES18 (146 amino-acid residues) or the nucD/regB phage lysozymes of *S. marcescens* (179 amino-acid residues) are more similar. ORF4 showed similarity to *E. coli* bacteriophage N15gp 55 protein, a protein of unknown function (Zimmer et al. 1998).

Located in the same orientation as the sep genes and 134bp downstream of the *SepC*

termination codon is a 204 base pair region assigned ORF5, which has high similarity to a *S. typhimurium* revolvase/invertase protein. However ORF5 is disrupted by two stop codons at amino-acid residues 19 and 64, making it unlikely that an active resolvase/invertase protein, is encoded by this region. A 256-bp region of encompassed by ORF5 (17498-17754) showed high similarity (77% identity) to the region (AF020806; 1629-1885 bp) encoding *S. typhimurium* DNA invertase gene (Valdivia et al. 1997) suggesting a similar ancestral origin.

Downstream of ORF5 and oriented in the opposite direction from 18935-18163 was a 870 base pair region of DNA designated ORF6 whose product showed high amino-acid similarity over two different reading frames to the insertion element *IS*91 of *E. coli* (Mendiola et al. 1992). The translated sequence is interrupted at amino-acid residue 149 of the *IS*91 element and later resumed on a second reading frame, before its similarity switched back to the original reading frame. Swtiching of ORF's is a common feature of members of the IS3 family where the transposase is encoded by this overlapping ORF's (Prere et al. 1990). However, the switch back to the initial strand is atypical. ORF6 may therefore be a dysfunctional relic of an ancestral *IS* element. It is unknown whether ORF6 contains a ribosomal binding site as its predicted location would lie outside the sequenced region. There was no DNA similarity to the *IS*91 element.

Analysis for protein motifs showed that a tripeptide cell-binding motif Asp-Gly-Arg (RGD), implicated in the binding of various adhesion proteins produced by parasites and viruses to eukaryotic cells (Leininger et al. 1991), is present in SepA and the *P. luminescens* TcdA, and TcaB proteins (Fig. 4). The RGD motif is present in cell surface adhesions produced by the human pathogen *Bordetella pertussis,* namely the filamentous heamagglutinin (220 kDa) (Relman et al. 1989), and the outer membrane protein pertactin (69 kDa) (Leininger et al. 1991). These motifs have been implicated in enhancing the binding of *B. pertussis* to eukaryotic cells. Because the RGD motif found in SepA falls in a

region of high similarity between SepA and its *P. luminescens* counterparts, it may play a role in meditating the attachment of the protein and/or the bacteria to the insect cell wall.

The hydropathicity profile of each of the Sep proteins was examined using the Kyte and Doolittle algorithm (Kyte and Doolittle, 1982) and compared to the relevant *P. luminescens* homologues. None of the Sep proteins contained a positively charged amino terminus followed by a hydrophobic region, characteristic of a signal sequence (Gierasch, 1989). The profiles of SepA, TcbA and TcdA were very similar (data not shown) and each exhibited a steep hydrophilic peak at the carboxyl terminus (residues 2055-2061 of SepA), specifically the protein sequence RRRRE (Fig. 4). Although both SepB and TcaC shared similarity to the *Salmonella* virulence protein SpvB, the amino-terminil of SepB and TcaC were hydrophilic as opposed to the hydrophobic nature of SpvB. The profile of SepC and its *Photorhabadus* counterpart TccC differed in that SepC had a slightly hydrophilic amino-terminus, whereas TccC lacked a hydrophilic amino-terminus and had a significantly hydrophobic carboxyl terminus from amino-acid residue 717 onwards (Fig. 3).

Analysis to detect repetitive motifs characteristic of the RTX family of toxins (Welch, 1991) using DOTPLOT showed only *P. luminescens* TccC contained a plot characteristic of a repeat motif present at the carboxy terminal (data not shown).

Analysis of DNA composition (%GC) and similarity

Comparisons of the GC content (Table 3) showed that the *SepA* and *SepB* genes were more GC-rich than their *P. luminescens* counterparts, while *SepC* and *tcaC* had similar GC content. The high GC content of *SepC* may be attributed to the close relationship of these protein products to the *rhs* family of wall-associated proteins which have a GC-rich core of 62% (Wang et al. 1998). Comparisons of the GC content of the *Sep* genes with that of the *S. entomophilia* genome shows that they are rather similar, suggesting that the *sep* genes were not recently acquired by *S. entomophilia*.

Identification of mini-*Tn10* location by sequence analysis

Analysis of the insertion points of the previously isolated mini-*Tn10* insertions (Fig. 2) within the putative ORF's (Table 4) revealed that ORF3 and ORF4 were interrupted by the -9, -23, -24 (ORF3) and -35 (ORF4) mutations. These insertions had no effect on the pathogenicity process, suggesting that ORF3 and ORF4 do not play a significant role in pathogenicity. However, the pADAP-35 mutation was at the 3' end of ORF4, resulting in the truncation of the final 11 amino-acid residues of ORF4 (Fig. 4), which may not have affected protein function. Further mutagenesis of ORF4 is therefore required to confirm that it has no role in pathogenicity. The mutations that caused loss of pathogenicity all resided within *SepA*, *SepB* or *SepC*. No mutation mapped to ORF1, ORF2 or ORF5.

Complementation analysis of the sep proteins

Following sequence data each of the *Sep* ORF's were excised as closely as possible with restriction enzymes, placed into pLAFR3 and placed in *trans* with the appropriate pADAP mutation. Complementation of *SepA* was undertaken through the use of the 8.5 kb *Hind*III clone (pMH45) which encompasses both ORF1 and *SepA*. *SepB* was excised as a 5.4 kb *Stu*I fragment and *SepC* was excised as a 4.6 kb fragment using one of the peripheral; *Bam*HI sites from the pBH32-13 mutation and the *Stu*I site of pBM32 (Fig. 2b).

Complementation analysis showed that pLAFR3 based *SepB* and *SepC* are able to complement their mutated pADK- counterparts. Grkovic et al. (1995) had already previously shown that *SepC* could complement itself. However, this was achieved through using the entire 11 kb *Hind*III, pGLA-20 fragment.

Whether *SepA* is able to complement itself has yet to be fully established. It was found that ~98% of the pMH45 construct was lost during the course of the bioassay. This latter result was sporadic and occasionally a repeated experiment would show the presence of diseased

grubs. Analysis of the macerates of these grubs showed that pMH45 was present indicating that pMH45 can possible complement *SepA*. However before further complementation analysis of *SepA* can be undertaken, measures to ensure the complementation plasmids stability are needed.

DISCUSSION

The large conjugative plasmid, pADAP, of *S. entomophilia* encodes the genes responsible for cessation of feeding and gut clearance, characteristics of amber disease in the New Zealand grass grub *C. zealandica.* This plasmid is present in all *S. entomophilia* and *S. proteamaculans* strains capable of causing amber disease (Glare et al. 1993) and had been implicated in disease processes (Grkovic et al. 1995). The applicant has defined a 16.9 kb region of kADAP that is sufficient to confer pathogenicity towards *C. zealandica* on pADAP-cured strains of *S. entomophilia* and on strains of *E. coli.* Hence, the region confers all the essential pathogenicity genes of *S. entomophilia* responsible for amber disease. Nucleotide sequence and mutagenesis analysis of the region revealed three genes, *SepA, SepB* and *SepC,* that together are sufficient for pathogenicity. Mutations in any of the three genes completely abolished the disease process and partial disease states were not detected, suggesting that the three genes may interact to exert an effect.

The 23-kb region cloned into pBR322 to make pBM32 conferred pathogenicity in pADAP-cured *S. entomophilia* strains with all symptoms of amber disease being observed. Insertion mutants in pBM32 that abolished pathogenicity were transferred to pADAP. The resultant strains showed a partial disease phenotype, including anti-feeding but not gut clearance, suggesting that an additional anti-feeding gene may be present elsewhere on pADAP. The occurrence of two different anti-feeding genes on pADAP also supports data of Grkovic et al. (1995) who found that suppression of feeding was stronger in the wild-type pADK-6 strain, compared to the partial disease state (pADK-10, pADK-13) of

inducing anti-feeding but no gut clearance. A putative anti-feeding gene, *amb2*, has already been isolated from the genomic DNA of *S. entomophilia* (Nunez-Valdez and Mahanty, 1996). Recent data indicate that the *amb2* locus resides at an as yet to be identified location on pADAP that is remote from the region identified herein (Hurst, unpublished data).

Sequence analysis and comparison of the products of the *sep* genes showed that they share significant similarity to the proteins TcbA (TcdA, TcaB, TccB), TcaC and TccC that comprise the toxin complexes of *P. luminescens*. Like the *P. luminescens* genes that *sep* genes of *Serratia* share a similar organisational pattern of three genes ordered in succession in the same orientation, and opposed by a terminil gene transcribed in the opposite direction. However, the order of sep genes differ, are slightly smaller in size, and comprise constituents of each of the *P. luminescens* loci tca (tcaB=sepA, tcaC=sepB), *luminescens* toxin gene tcd (Ensign et al. 1997) is also similar to SepA. The similarity shared between the *sep* and *tc* gene products suggests that they are members of a new family of insecticidal toxins. The lack of DNA similarity as opposed to protein similarity between *sep* and *P. luminescens* tc genes together with the differnce in GC content of the *sepA* and *sepB* genes compared to the tc genes, suggests that these genes were present in the common enterobacterial ancestor of *P. luminescens* and *S. entomophilia* and were not acquired by a more recent horizontal transfer event.

The *Photorhabadus* toxins were isolated as a composite of proteins which are hypothesised to interact synergistically to form a toxin complex. The toxins are also able to exert an anti-feeding effect (Bowen et al. 1998; Bowen and Ensign, 1998). This is consistent with the results we obtained with the *sep* mutants. pADAP-cured *S. entomophilia* strains containing the pathogenicity clone pBM32 exert an anti-feeding effect on the grass grub and individual mutations within any of the *sep* genes have an identical phenotype, completely abolishing pathogenicity. The *Photorhabadus* toxins have a wide host range, affecting Lepidoptera, Coleoptera and Dictyoptera and undergo post translational

proteolytic processing (Bowen et al. 1998). No similarities of *sep* proteins were found to the *Photorhabadus* toxin component TccA, and only the amino-terminus of TcaA shared similarity to *SepA*. This and the difference in the hydrophobicity profiles of *SepC* and TccC, may account for specificity of the *sep* proteins towards *C. zealandica.* However the *sep* proteins have yet to be purified and it is unknown whether the *sep* genes are expressed when *S. entomophilia* is ingested by other insects. Therefore the possibility that these newly-described toxins may exhibit a broader host range cannot be ruled out.

The *Photorhabdus* toxin TcbA shares weak similarity to the *Clostridium difficile A* and *B* toxins (Bowen, 1998), but no such similarities were found to *SepA*. *C. difficile A* and *B* toxins belong to the RTX (repeats in toxin) family of toxins which are noted for the presence of several carboxyl terminal repeats (von Eichel-Streiber et al. 1992). A search of the *sep* proteins and their *P. luminescens* homologues for protein repeats showed that only the *P. luminescens* TcaC protein contained a repeat-type signature. The TcaC carboxy-terminal repeat bears little resemblance in size or number of repeats found in RTX toxins (von Eichel-Streiber et al. 1992). *SepA* does not show weak similarity to the mosquitocidal toxin Cbm71 of *C. bifermentans* (Barloy et al. 1996). However when this region is compared with the relevant *Photorhabdus* homologues, it is a region with little similarity.

SepB has strong similarities to both *P. luminescens* TccC and the *Salmonella* virulence gene product SpvB (Gulig et al. 1992). SpvB is believed to enhance the survival of virulent *Salmonella* in macrophages (Libby et al. 1997). It has been suggested that TcaC may act by attacking insect haemocytes (Bowen et al. 1998). However, haemocytes reside within the insect haemocoel and *S. entomophilia* does not invade the haemocoel until late in the infection process (Jackson et al. 1993), suggesting that *SepB* may act in some other way. The similarity of *SepB* and TcaC is high to SpvB but diminishes ten amino-acid residues upstream of the proline-rich region found in SpvB that is postulated to divide the protein into separate domains (Roudier et al. 1992). This may indicate a vital role for the amino-

terminus of both *SepB* and SpvB in interacting with an evolutionarily-conserved eukaryotic protein.

The *SepC* protein shows high similarity to a family of cell wall-associated bacterial proteins such as the *B. subtilis* wall-associated protein (WAPA) and members of the *E. coli* rhs element family. The function of the Rhs proteins has yet to be established, but they are believed to be cell surface ligand-binding proteins (Hill et al. 1994). The Rhs proteins and the *B. subtilis* was-associated protein contain a characteristic repetitive peptide motif, but no such motif was observed in *SepC*. A feature of rhs elements is the presence of a downstream IS element (Wang et al. 1998). A degenerate IS91-type transposase element (ORF6) is present downstream of *SepC*. The IS91 element has been found associated with plasmids or chromosomal genes involved in α-haemolysin synthesis, and has been postulated to play a pivotal role in the spread of the α-haemolysin genes by means of the IS91-mediated recombinational activity (Zabala et al. 1984). It seems possible an IS element adjacent to *SepC* may have been involved in the acquisition of the *sep* genes by *S. entomophilia.*

Blackburn et al. (1998) undertook histological examinations of the lepidopteran *Manduca sexta* after treatment with the *P. luminescens* Tca toxin complex introduced by feeding or haemcoelic injection. They found blebbing of the midgut epithelium into the lumen, resulting in lysis and formation of cavities. Similar histological studies have been undertaken at various stages throughout the infection cycle of *S. entomophilia* in *C. zealandica,* and reveal a visible deterioration in the number of fat cells to almost minimal levels, and an emptying of the larval gut. However no blebbing of the midgut epithelium was observed (Jackson et al. 1993).

The *S. entomophilia* pathogenicity region endows pathogenicity on members of the Enterobacteraceae such as *Klebsiella spp., Enterobacter agglomerans, E. coli,* and *Serratia*

species (Glare et al. 1996). From this we can infer that the *Sep* proteins are the major virulence determinants, that the promoters of the *sep* genes are expressed constitutively or under the control of conserved regulatory genes, or a negative regulatory gene present in the pathogenicity region, and that export of the toxin proteins is carried out by a conserved chromosomally encoded system, or is an intrinsic property of the *sep* proteins. The *Sep* proteins have no obvious amino terminal signal sequences, a facet shared with E-Group colicins. The release of cloacin DF13 is mediated through a small lipoprotein designated BRP, for bacteriocin-release protein. Low level expression of BRP in conjunction with phospholipase A leads to the release of cloacin DF13, along with bacterial periplasmic proteins. However if expressed in high amounts, BRP causes cell death by cell lysis (vad der Wal, 1998). The close proximity and similar orientation pattern of ORF3 to the *sep* genes indicate that ORF3 may have an as yet to be determined important functional role. Protein similarity searches show that it has high similarity to the bacteriophage lysozyme family. In relation to amino-acid size, ORF3 closely resembles the LZBP22 lysozyme of the *Salmonella* P2 bacteriophage, a protein essential for the lysis of the bacterial cell wall (Rennell and Poteete, 1985). It is possible that ORF3 may facilitate the release of the *sep* proteins by lysing the bacterial cell wall. A low level expression of ORF3 might, as in the case of BRP, allow the passage of the *sep* proteins across the cell wall without causing cell death. The reason that the pBM32-9 and -24 mutations were unable to abolish the disease process could be due to a masking of ORF3 function by natural cell lysis of the bacteria.

A region of repetitive DNA was identified between nucleotides 683 to 743, centered within a 1.2-kb AT rich stretch of DNA that contains no potential ORF's. The repeat motif is flanked by an upstream 13-bp palindrome and a degenerate downstream 33-bp palindrome. Repeats have been found to be common sites for recombination (Allgood et al. 1988), or to facilitate the binding of proteins. A 66-bp DNA sequence termed the *rsk* element for reduced serum killing, of the *S. typhimurium* 95-kb virulence plasmid, comprises of a series

of direct 10-bp repeats with a 21-nucleotide periodicity. The *rsk* element is believed to titrate out a *trans*-acting factor, enhancing the expression of the *Salmonella* serum resistance gene (Vandenbosch et al. 1989). It is not known whether these repeats and/or flanking palindromes have a role in the pathogenicity process. The deletion derivative pAC24, which encompasses this region, was still pathogenic towards the grass grub. However, this deletion could also unknowingly remove the complete regulatory circuit of the pathogenicity region, leading to constitutive expression.

THE ARABINOSE EXPRESSION SYSTEM

Methodology

Using the polymerase chain reaction (PCR) the initiation codon ATG of the three *sep* genes (*sepA*, *sepB* and *sepC*) were individually placed into the unique *Nde*I site (restriction enzyme site CATGG) of the HIS-tag arabinose expression vector pAV2-10 (obtained from Chuck Shoemaker –AgResearch). Because large proteins i.e. greater than 50 kda are limited in their ability to bind to HIS tag affinity columns the carboxyl terminus of each of the Sep proteins did not need to be in frame with the HIS-tag site. Instead wild type DNA (non PCRd) containing a downstream chloramphenicol resistance gene was ligated into the appropriate restriction enzyme site (*sepA Sun*I; *sepB Hin*dIII; *sepC Bst*XI) of the pAV2-10-*sep* derived vectors:-

-the use of the chloramphenicol resistant marker provided by the vector pACYC184 enhances the stability to each of the expression constructs i.e. -the antibiotic ampicilin to which the pAV2-10 is resistant too is cleaved in the media to an inactive form leading to possible plasmid free segregants arising. Conversely the antibiotic chloramphenicol is not cleaved heightening the level of plasmid stability under conditions of arabinose induction.

To validate the legitimacy of the fused genes to the arabinose expression vector, PCR generated products and the ligation junctions were verified by DNA sequencing.

Concurrent to this the *sepB* and *sepC* genes were placed as derived from pADAP downstream of *sepA*. Also *sepA*, *sepB* and *sepC* were placed as in pADAP downstream of orf3. This simulated wildtype conditions (i.e. the arrangement of the *sep* genes on pADAP) and hopefully get the production of the *sep* genes and the complex driven off the one upstream promoter. A method which Western analysis has shown to be successful –with moderate levels of *sepA*, *sepB* and *sepC* being detected.

The arabinose expression system is one of the tightest systems known with almost complete abolition of gene product under arabiniose free conditions Guzman *et al.* (1995), this abolition can be enhanced by providing glucose to the medium. In contrast providing arabinose at the concentration of 0.2% will switch the arabinose promoter on express any genes under its control e.g. *sepA* etc. Typically an overnight culture of the *E. coli* strain was set up the next day an 100 µl of the culture was suspended in fresh media supplemented with chloramphenicol (30 µg/ml) the culture was grown until an OD of 400 at which time arabinose was added to the culture to a final concentration of 0.2% and the culture left shaking at 30 °C for 18 hours.

To date Western analysis has shown that each of the proteins is expressed and expressed to its correct predicted size:

SepA 262.7 kdal

SepB 156.6 kdal

SepC 107 kdal

SepC is expressed at high levels with minor levels of proteolytic cleavage. However both SepA and SepB though expressed are cleaved in high amounts by endogenous *E. coli* proteases. Alternative strains of *E. coli* are going to be assessed for loss of proteolytic activity against SepA and SepB

It has also been shown that placing all three of the *sep* genes under the control of a single arabinose promoter will result in the production of basil levels of the SepA, SepB, SepC toxin complex.

Each of the following Coleopteran species were mouth injected with 3-5 µl of an overnight suspension of induced bacteria (*E. coli* strain DHB101) containing either SepA, SepB and SepC or orf3, SepA, SepB and SepC.

Each larvae was then given a $3mm^3$ piece of carrot coated with a 50% solution (dH_20) of arabinose. Observations were noted each day and the larvae refed with a $3mm^3$ piece of carrot coated with a 50% solution (dH_20) of arabinose

Red headed cock chaffer

Tasmanian grass grub

Odontara

Grass grub (positive control)

Under these conditions it has been found that the arabinose expressed toxin complex SepA, SepB and SepC is active against grass grub but not any of the other species of scarabs tested (see above). It is therefore thought unlikely that the toxin complex will have activity to other insect orders.

SUMMARY

The bacteria *Serratia entomophilia* and *S. proteamaculans* cause amber disease in the grass grub, *Costelytra zealandica* (Coleoptera: Scarabaeidae), an important pasture pest in New Zealand. Larval disease symptoms include amber colouration, clearance of the gut and rapid cessation of feeding, before eventual death. The region containing pathogenic determinants of the disease has been cloned, and further defined by mutagenesis and deletion analysis to a 16.9 kb region. Sequence analysis of the minimal pathogenic encoding region showed significant protein homology, but little sequence homology to a group of newly described toxins from a member of the Enterobacteriaceae, *Photorhabadus luminescens*. This pathogenicity-encoding region from *S. entomophilia* plasmid pADAP is the subject of the invention. The proteins encoded by the genes (*sepA, sepB, sepC)* within the 16.9 kb region can be used for insect control whether as an inundative pesticide, within baits or expressed in other organisms such as plants or microbes.

Aspects of the present invention have been described by way of example only and it should be appreciated that modifications and additions may be made thereto without departing from the scope thereof as defined in the appended claims.

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Table 1 Bacterial strains, plasmids and bacteriophage used in the study

Bacteria	Description	Reference
Escherichia coli		
DH5α	F⁻ ϕ80d *lacZ*ρM15 ρ(*lac*ZYA-*arg*F)U169 *rec*A1 *end*A1 supE44	Hanahan (1983)
DH10B	F⁻ *mcr*A ρ(*mrr-hsd*RMS-*mcr*BC)ϕ80d *lacZ*ρM15 ρ*lacX74 endA1 recA1 deo*Rρ(*ara, leu*) 7697 *ara*D139 *gal*U *gal*K *nup*G *rps*L λ⁻.	Lorow and Jessee, (1990)
DF1	γδ transposase(*tnpA*)	Gibco BRL
MC1061	*sup*° *hsdR mcrB araD*139 ρ(*araA BC-leu*)7679 ρ*lac*X74 *galU galK rpsL thi*	Casadaban and Cohen, (1980)
MC4100	*araD139* ρ(*lac*ZYA-*argF*)U169 *rpsL150* St^R *relA1 flbB5301 deoC1 ptsF25 rbsR*	Silhavy *et al.* (1984)
XL1-BlueMRA	ρ(*mcrA*)183 ρ(*mcrCB-hsdSMR-mrr*)*173 endA1 supE44 thi-1 reA1 gyrA96 relA1*	Stratagene
Serratia entomophila		
A1MO2	Ap^R, pADAP, pathogenic.	Grimont *et al.* (1988)
5.6	heat cured pADAP minus derivative of A1MO2	Glare *et al.* (1993)
5.6RC	Cm^R *recA⁻* pADAP minus strain	Grkovic *et al.* (1996)
5.6RK	Kn^R *recA⁻* pADAP minus strain	this study
Plasmids		
pACYC184	Cm^R Tc^R	Chang and Cohen, (1978)
pADAP	Amber disease associated plasmid	Glare *et al.* 1993)
pBR322	Ap^R, Tc^R	Bolivar *et al.* (1977)
pBM32	23-kb *Bam*HI fragment from pMH32 cloned in pBR322	this study
pBM32-1-40	pBM32 containing mini-*Tn10* insertions	this study
pDELTA1	Ap^R, Sm^R, Kn^R, $sucrose^R$	Gibco BRL
pLAFR3	Tc^R pRK290 with λ*cos*, *lacZα* and multi-cloning site from pUC8.	Staskawicz *et al.* (1987)
pRK2013	IncP, Kn^R Tra RK2 *repRK2 repE1*	Ditta *et al.* (1980)
pGLA20	10.6-kb *Hin*dIII pADAP fragment cloned in pLAFR3	Corbett (unpublished)
pACρ4	19-kb *Bam*HI fragment from pBM32-4 cloned in pACYC184	this study
pACρ8	17-kb *Bam*HI fragment from pBM32-8 cloned in pACYC184	this study
pACρ10	19.5-kb *Bam*HI fragment from pBM32-10 cloned in pACYC184	this study
pACρ20	20-kb *Bam*HI fragment from pBM32-20 cloned in pACYC184	this study
pACρ23	21-kb *Bam*HI fragment from pBM32-23 cloned in pACYC184	this study
pACρ24	21.2-kb *Bam*HI fragment from pBM32-24 cloned in pACYC184	this study
pADK-10	pADAP::mini-Tn*10* insertion in 10.6-kb *Hin*dIII fragment, Kn^R non-pathogenic	Grkovic *et al.* (1995)
pADK-13	pADAP::mini-Tn*10* insertion in 10.6-kb *Hin*dIII fragment, Kn^R non-pathogenic	Grkovic *et al.*(1995)
pADK-35	pADAP::mini-Tn*10* insertion in 10.6-kb *Hin*dIII	Grkovic *et al.* (1995)

	fragment, Kn^R, pathogenic	
pMH32	23-kb *Bam*HI frgament of pADAP cloned into pLAFR3	this study
pMH41	33-kb *Bam*HI fragment of pADAP cloned into pLAFR3	this study
pBM32	23-kb *Bam*HI fragment of pMH32 cloned into pBR322	this study
pUC19	Ap^R, *lacZα*, multi-cloning site	Yannish-Perron, *et al.* (1985)
Bacteriophage		
λNK1316	mini-Tn*10* derivative 103 donor λb522 c1857 Pam80 nin5	Kleckner *et al.* (1991)

Table 2 Position of genes and features of the predicted gene products encoded by *sep* genes

ORF	Putative ribosome-binding site*	Longest potential coding region		*sep* %GC (*P. luminscens* homologue, %GC)
		Start at nucleotide	Stop at nt (ORF size bp)	
sepA	ATGGGACCATCAACGTAATGAA TGAGG	2413	9547 (7131)	54 (*tcbA*, 43; *tcdA*, 44)
sepB	CGAGGAGACTGAGCATGCAA	9598	13885 (4287)	58 (*tcaC*, 51)
sepC	ACAGGAGATCACATGAGC	14545	17467 (2922)	55 (*tccC*, 54)
ORF1	CATAGAGACTGTCGCTATGTTA	1287	1587 (300)	39
ORF2	TTGGAGAATAACCGCCATGTT	1590	1863 (273)	39
ORF3	GGGGGAGAAAAATGAAG	1860	2294 (435)	51
ORF4	TGACTGGGAAGGAGGGGGGAC GGTGATGAGT	13908	14483 (576)	60
ORF5	TAACGAGACTTTTTAGCAAAAT GGCACTTT	1761-1755, 1755-1773		?
ORF6	GAGCATGGC-Mini-Tn*10*-8♦	18934-18064		?

* Putative ribosome-binding sites are underlined, and potential start codons are in boldface; nt, nucleotides; ? degenerate or incomplete ORF. ♦ ORF transcribed in opposing direction.

Table 3. **Comparisons of GC content between the *Sep* and *P. luminescen* genes**

Sep (%GC)	*P. luminescen* toxin (%GC)
sepA (54%)	*tcbA* (43%) *tcdA* (44%)
sepB (58%)	*tcaC* (51%)
sepC (55%)	*tccC* (54%)

Table 4. **Similarities of products of putative ORF's to protein sequences in the database detected using BlastP**

ORF (a.a size)	Protein homo-logue (a.a size)	Degree of similarity %identity/%similarity (over) a.a residue – a.a residue	Function of the homologous protein	Organism	Blast score Reference■
SepA (2373)	TcbA (2504)	34/50 (1675) 41-1628* 57/72 (751) 1630-2374*	insecticidal toxin complex protein	*Photorhabdus luminescens*	0.0 AF047457
	TcdA (2405)	40/55 (2458)*	insecticidal toxin complex protein	*P. luminescens*	0.0 Ensign *et al.*, (1997)
	TcaB (1189)	38/54 (764) 1625-2374* 29/50 (281) 936-1198*	insecticidal toxin complex protein	*P. luminescens*	e^{-137} AF046867
	TccB (1565)	36/51 (859) 1575-2373* 31/51 (289) 930-1204*	insecticidal toxin complex protein	*P. luminescens*	e^{-136} AF047028
	TcaA (1095)	36/56 (90) 94-183* 18/39 (530) 435-928*	insecticidal toxin complex protein	*P. luminescens*	$1e^{-8}$ AF046867
	TccA (965)	27/45 (186) 115-280*	insecticidal toxin complex protein	*P. luminescens*	$5e^{-6}$ AF047028
	Cbm71 (613)	24/41 (199) 1057-1250*	Mosquitocidal toxin Cbm71	*Clostridium bifermentans*	g2127309
SepB (1428)	TcaC (1485)	49/63 (1276)1-1263* 64/78 (152) 1270-1421*	insecticidal toxin complex protein	*P. luminescens*	0.0 AF046867
	SpvB (591)	40/52 (357) 9-365*	*Salmonella* virulence protein	*Salmonella typhimurium*	$4e^{-62}$ S22664
SepC (938)	TccC (1043)	53/66 (836) 3-782*	insecticidal toxin complex protein	*P. luminescens*	0.0 AF047028
	SC2H4.02 (2183)	23/34 (639) 68-677*	Hypothetical wall associated protein	*Streptomyces coelicolor*	$2e^{-12}$ AL031514.1
	WapA (2334)	22/34 (430) 255-677* 20/36 (613) 48-625*	Wall associated protein Precursor	*B. subtilis*	$2e^{-5}$ S32920
	Y15898 (334)	21/34 (542) 181-684*	hypothetical wall associated protein	*Coxiella burnetii*	$9e^{-5}$ Y15898
	Rhs core (1420)	21/35 (463) 237-677* 21/36 (285) 35-300*	Rhs core protein	*E. coli*	$3e^{-4}$ AF044501
ORF3 (144)	BB103G (263)	45/62 (142) 1-139*	morphogenesis protein of bacteriophage B103	*Bacillus subtilis*	$3e^{-27}$ CAA67646
	LZBP22 (146)	46/61 (139) 1-143	Phage P22, lysozyme (E 3.2.1.17)	*Salmonella*	$1e^{-24}$ gi 138699
ORF4 (191)	Gp55 (181)	28/42 (188) 1-184*	bacteriophage N15 protein	*E. coli*	$1e^{-6}$ AF064539
ORF5 (236)	SprA	75/79(68) 1-68 ♦	Resolvase/invertase homologue	*S. typhimurium*	$7e^{-19}$ AF029069 AF020806
ORF6 (310)	IS*91*	39/56 (94) 130-197♦ ⁻1• 39/58 (94) 224-318♦ ⁻2• 30/48 (76) 319-395♦ ⁻1•	IS*91* transposase	*E. coli*	$4e^{-28}$ S23782

Percent identities and similarities were calculated in relation to the deduced gene products of the sequenced ORF. *indicates position of amino-acid similarity in relation to sequence generated in this study. ♦ indicates position of amino-acid similarity in relation to data base protein sequence. • reading frame. ■ similarities were considered potentially significant if the BlastP score exceeded e^{-5}.

Table 5 **Positions of mini-Tn*10* insertions**

Mini-Tn*10* insertion #	**ORF**	**Position downstream of initiation codon (bp)**
9/23	ORF3	120
24	ORF3	345
4	*sepA*	747
27	*sepA*	1037
40	*sepA*	1097
6	*sepA*	1727
38	*sepA*	2887
2	*sepA*	3197
5	*sepA*	3737
3	*sepA*	3697
19	*sepA*	3697
30	*sepA*	4467
37	*sepA*	4467
31	*sepA*	4627
12	*sepB*	182
22	*sepB*	172
11	*sepB*	362
10	*sepB*	2162
35	ORF4	557
13	*sepC*	2525
8		18937
ORF4/-35 junction GGG CGC *<u>TGA</u>* *<u>TGA</u>* *ATC*		

THE CLAIMS DEFINING THE INVENTION ARE:

1. A purified and isolated nucleic acid molecule comprising a nucleotide sequence of SEQ ID NO: 1 that encodes at least one of:

   (i) an insecticidal protein complex, or

   (ii) a functional fragment of said complex, or

   (iii) a neutral mutation of said complex, or

   (iv) a homolog of said complex,

   each of which have at least 75% nucleic acid homology to SEQ ID NO: 1 and are capable of hybridising with said nucleic acid molecule under stringent hybridisation conditions.

2. A purified and isolated nucleic acid molecule as claimed in Claim 1 comprising the nucleotide sequence 1995-18937 of SEQ ID NO: 1.

3. A purified and isolated nucleic acid molecule as claimed in Claim 1 comprising one or more of the nucleotide sequences 2411-9547, 9589-13883 or 14546-17467 of SEQ ID NO: 1.

4. A purified and isolated nucleic acid molecule as claimed in Claim 3 comprising all of nucleotide sequences 2411-9547, 9598-13884 and 14546-17467 of SEQ ID NO: 1.

5. A purified and isolated nucleic acid molecule as claimed in Claim 1 comprising a sequence of SEQ ID NO: 1, operably linked to at least one further nucleotide sequence which encode an insecticidal protein.

6. A purified and isolated nucleic acid molecule as claimed in Claim 2 comprising nucleotides 1955-18937 of SEQ ID NO: 1, operably linked to at least one further nucleotide sequence which encode an insecticidal protein.

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7. A purified and isolated nucleic acid molecule as claimed in Claim 3 comprising a sequence of SEQ ID NO: 1,or one or more of nucleotides 2411-9547, 9598-13884 or 14546-17467 of SEQ ID NO: 1, operably linked to at least one further nucleotide sequence which encode an insecticidal protein.

8. A purified and isolated nucleic acid molecule as claimed in any one of claims 4 through 6 wherein the said nucleotide sequence includes the nucleotide sequence which codes for at least one of the *Bacillus* delta endo toxins, vegatative insecticidal proteins (vips), cholesterol oxidases, *Clostridium bifermentens* mosquitocidal toxins and/or *Photorhabadus luminescens* toxins.

9. A purified and isolated nucleic acid molecule as claimed in claim 1 wherein nucleic acid molecule may comprise DNA, cDNA or RNA.

10. A purified and isolated nucleic acid molecule as claimed in claim 1 wherein the nucleic acid molecules said fragment, neutral mutation or homolog thereof capable of hybridising to said nucleic acid molecule, hybridise to the nucleotide sequence of SEQ ID NO: 1, or nucleotides 1955-18937, 2411-9547, 9598-13884 or 14546-17467 of SEQ ID NO: 1 if there is at least 75% or greater identity between the sequences.

11. A purified and isolated nucleic acid molecule as claimed in claim 1 wherein the nucleic acid molecule may be isolated from *Serratia entomophila* or *Serratia proteamaculans* strains of bacteria.

12. A recombinant expression vector(s) containing the nucleic acid molecule as claimed in Claim 1 and host transformed with the vector expressing a polypeptide.

13. A recombinant expression vector(s) as claimed in claim 11 wherein the vector is selectable from any suitable natural or artificial plasmid/vector.

14. A recombinant expression vector(s) as claimed in claim 13 wherein said suitable natural or

artificial plasmid/vector, including, pUC 19 (Yannish-Perron et al. 1995), pProEX HT (GibcoBRL, Gaithersburg, MD, USA), pBR322 (Bolivar et al. 1977), pACYC184 (Chang et al. 1978), pLAFR3 (Staskowicz et al. 1987).

15. A polypeptide resulting from the transformation or transfection of a host cell with a recombinant expression vector as claimed in any one of Claims 12 through 14.

16. A method of producing a polypeptide of claim 15 comprising the steps of:

(a) culturing a host cell which has been transformed or transfected with said vector as defined above to express the encoded polypeptide or peptide; and

(b) recovering the expressed polypeptide or peptide.

17. The use of a ligand that binds to a polypeptide of claim 15 to isolate and/or identify the polypeptide of claim 15.

18. An antibody or antibody binding fragment that binds to a polypeptide of claim 15.

19. Probes and primers comprising a fragment of the nucleic acid molecule as claimed in Claim 1 wherein said fragment is hybridisable under stringent conditions to a native insecticidal gene sequence.

20. Probes and primers comprising a fragment of the nucleic acid molecule as claimed in claim 19 wherein said probes and primers enable the structure and function of the gene to be determined and homologs of the gene to be obtained from bacteria other than *Serratia* sp.

21. A polypeptide as claimed in Claim 15 wherein the polypeptide has insecticidal activity encoded by the nucleic acid molecule of claim 1, or a functional fragment, neutral mutation or homolog thereof.

22. A polypeptide having insecticidal activity as claimed in claim 21 wherein the polypeptide

comprises the amino acid sequence of SEQ ID NO: 1 or a functional fragment, neutral mutation or homolog thereof.

23. A polypeptide having insecticidal activity as claimed in claim 21 wherein the polypeptide comprises amino acids 32-5118 of SEQ ID NO: 1.

24. A polypeptide having insecticidal activity as claimed in claim 21 wherein the polypeptide comprises at least one amino acid sequence of SEQ ID NO: 2; SEQ ID NO: 3; SEQ ID NO: 4; SEQ ID NO: 5 or SEQ ID NO: 6.

25. A polypeptide having insecticidal activity as claimed in claim 24 wherein the polypeptide preferably comprises amino acid sequence SEQ ID NO: 4; SEQ ID NO: 5 and SEQ ID NO: 6.

26. A polypeptide having insecticidal activity as claimed in claim 24 wherein the polypeptide preferably comprises all of SEQ ID NOs: 2-6.

27. A polypeptide having insecticidal activity as claimed in claim 21 wherein the polypeptide is obtained by expression of a DNA sequence coding therefore in a host cell or organism.

28. A polypeptide having insecticidal activity as claimed in claim 27 wherein the polypeptide comprises the amino acid sequence of SEQ ID NO: 1 linked to at least one further amino acid sequence encoding an insecticidal protein.

29. A polypeptide having insecticidal activity as claimed in claim 28 wherein the at least one further amino acid sequence includes the amino acid sequence which codes for *Bacillus* delta endo toxins, vegatative insecticidal proteins (vips), cholesterol oxidases, *Clostridium bifermentens* mosquitocidal toxins and/or *Photorhabadus luminescents* toxins.

30. A polypeptide having insecticidal activity as claimed in claim 28 wherein the polypeptides comprise at least 50%, preferably 60%, more preferably 70% and most preferably 90-95% or greater identity to SEQ ID NO: 1.

31. A polypeptide having insecticidal activity as claimed in claim 21 wherein the polypeptide is produced by expression of a vector comprising the nucleic acid of SEQ ID No:1 or a functional fragment, neutral mutation or homolog thereof, in a suitable host cell.

32. An insecticidal composition comprising at least the polypeptide as claimed in claim 21 and an agriculturally acceptable carrier.

33. An insecticidal composition as claimed in claim 32 wherein more than one polypeptide is included in the composition.

34. An insecticidal composition as claimed in claim 32 or 33 wherein the composition comprises additional pesticides, including compounds known to possess herbicidal, fungicidal, insecticidal or nematicidal activity.

35. An insecticidal composition as claimed in claim 34 wherein the composition comprises other known insecticidally active agents, including *Bacillus* delta endo toxins, vegatative insecticidal proteins (vips), cholesterol oxidases, *Clostridium bifermentens* mosquitocidal toxins and/or *Photorhabadus luminescents* toxins.

36. A method of combating pests, said method comprising applying to a locus, host and/or the pest, an effective amount of the polypeptide as claimed in Claim 21 that has functional insecticidal activity against said pest.

37. A method of inducing amber disease or like condition in insects comprising delivery to an insect an effective amount of the polypeptide as claimed in Claim 21 that has functional insecticidal activity against said insect.

38. A method of inducing amber disease or like condition in insects as claimed in claim 37 comprising delivery to an insect an effective amount of the polypeptide wherein the insect is selected from the order comprising Coleoptera.

39. A method of inducing amber disease or like condition in insects as claimed in Claim 38

comprising delivery to an insect an effective amount of the polypeptide wherein the insect includes *Costelytra zealandica* (Coleoptera: Scarabaeidae).

40. A method of delivering the insecticidal polypeptide to induce amber disease or like condition in insects including delivery of the insecticidal polypeptide as claimed in Claim 39 to the insect by any one of presenting the insecticidal polypeptide orally as a solid bait matrix, as a sprayable insecticide sprayed onto a substrate upon which the insect feeds, applied directly to the soil subsurface or as a drench or is expressed in an transgenic plant, bacterium, virus or fungus upon which the insect feeds.

41. A transgenic plant, bacterium virus or fungus, incorporating in its genome, a nucleic acid molecule as claimed in Claim 1 for providing the plant, bacterium virus or fungus with an ability to express an effective amount of an insecticidal polypeptide.

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(54) Title: NUCLEOTIDE SEQUENCES ENCODING AN INSECTIDAL PROTEIN COMPLEX FROM SERRATIA

(57) Abstract: The present invention concerns novel nucleotide sequences encoding proteins from the Enterobacteriaceae, *Serratia entomophila* and *Serratia proteamaculans*, and the use of said nucleotide sequences and proteins for inherent insecticidal and potentially metazoacidal properties. The invention relates to an isolated nucleic acid molecule comprising a nucleotide sequence that encodes an insecticidal protein complex, or a functional fragment, neutral mutation, or homolog thereof capable of hybridising with the nucleic acid molecule under standard hybridisation conditions. The nucleotide sequences include a pathogenicity-encoding region cloned from bacteria *Serratia entomophilia* and *S. proteamaculans*. The region contain pathogenic determinants of a disease that affect the grass grub, *Costelytra zealandica Coleoptera: Scarabaeldae*, an important insect pasture pest in New Zealand. The proteins encoded by determined genes may be used for insect control whether as an inundative pesticide, within baits or expressed in other organisms such as plants or microbes.

FIGURE 1

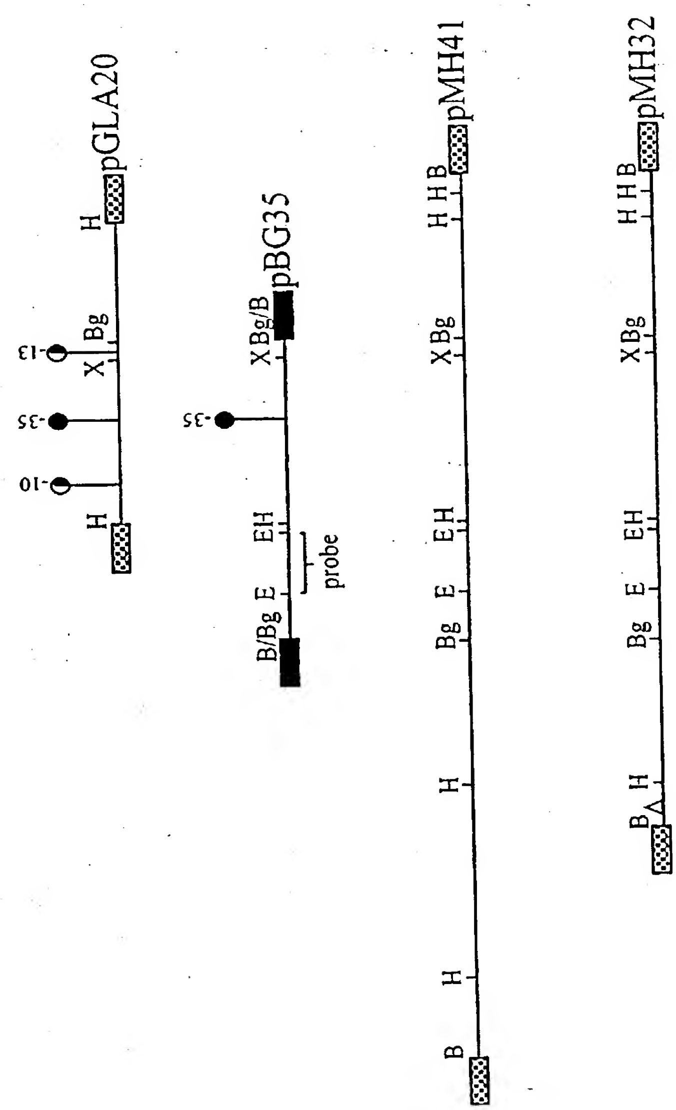
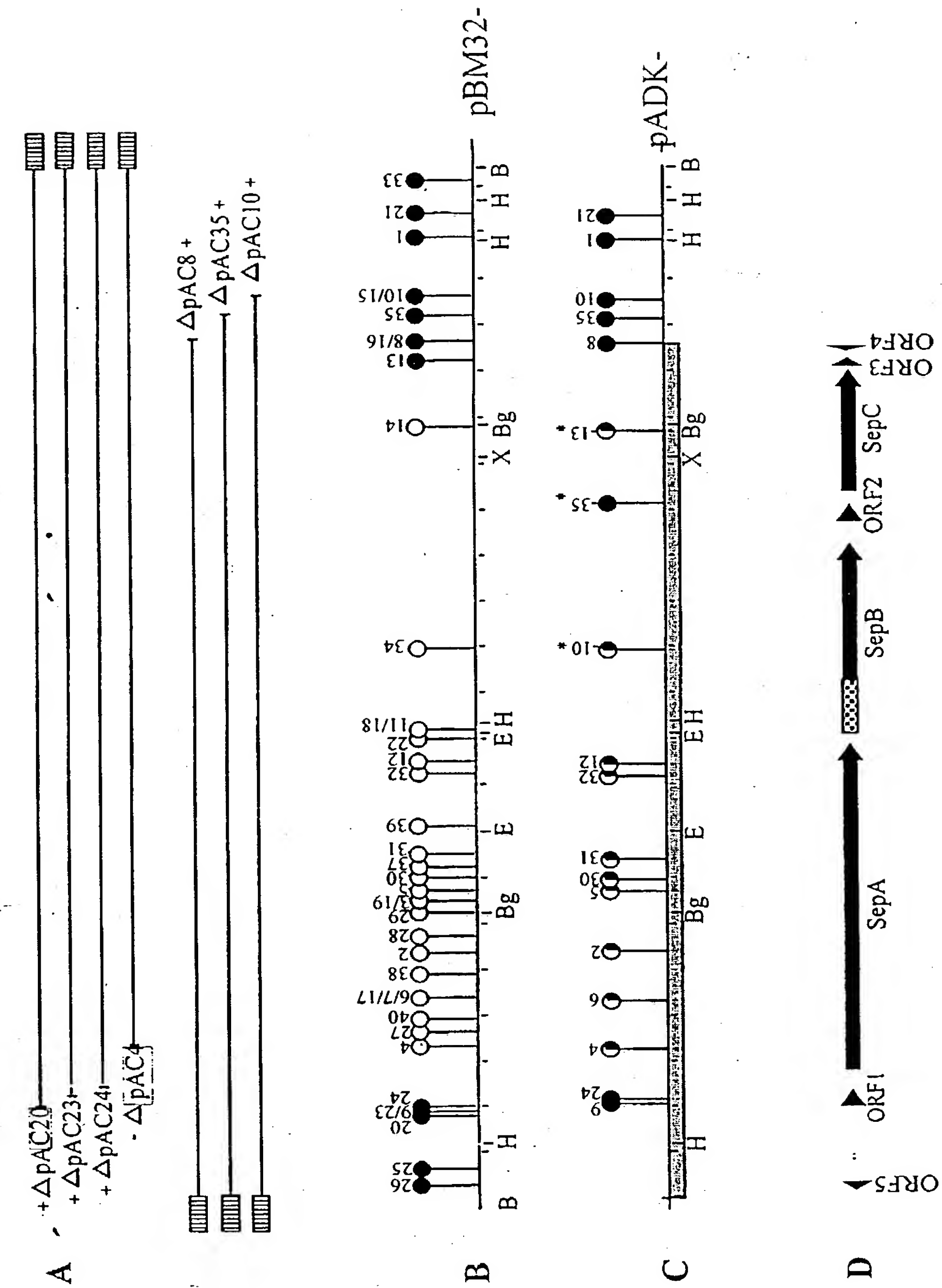


FIGURE 2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ΔpAC24
ΔpAC4
ΔpAC8
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ΔpAC10
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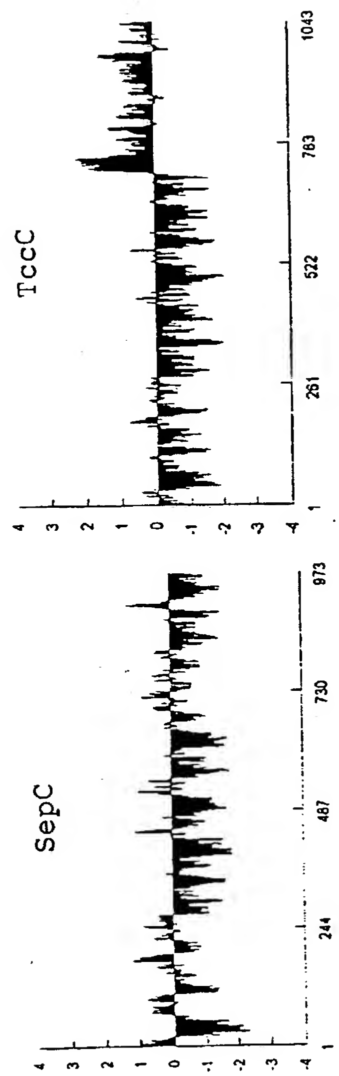
C
pADK-
D
ORF1
SepA
SepB
ORF2
SepC
ORF3
ORF4
ORF5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2
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-3
-4
1
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487
730
973
TccC
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1
0
-1
-2
-3
-4
1
261
522
783
1043

FIGURE 3

FIGURE 4

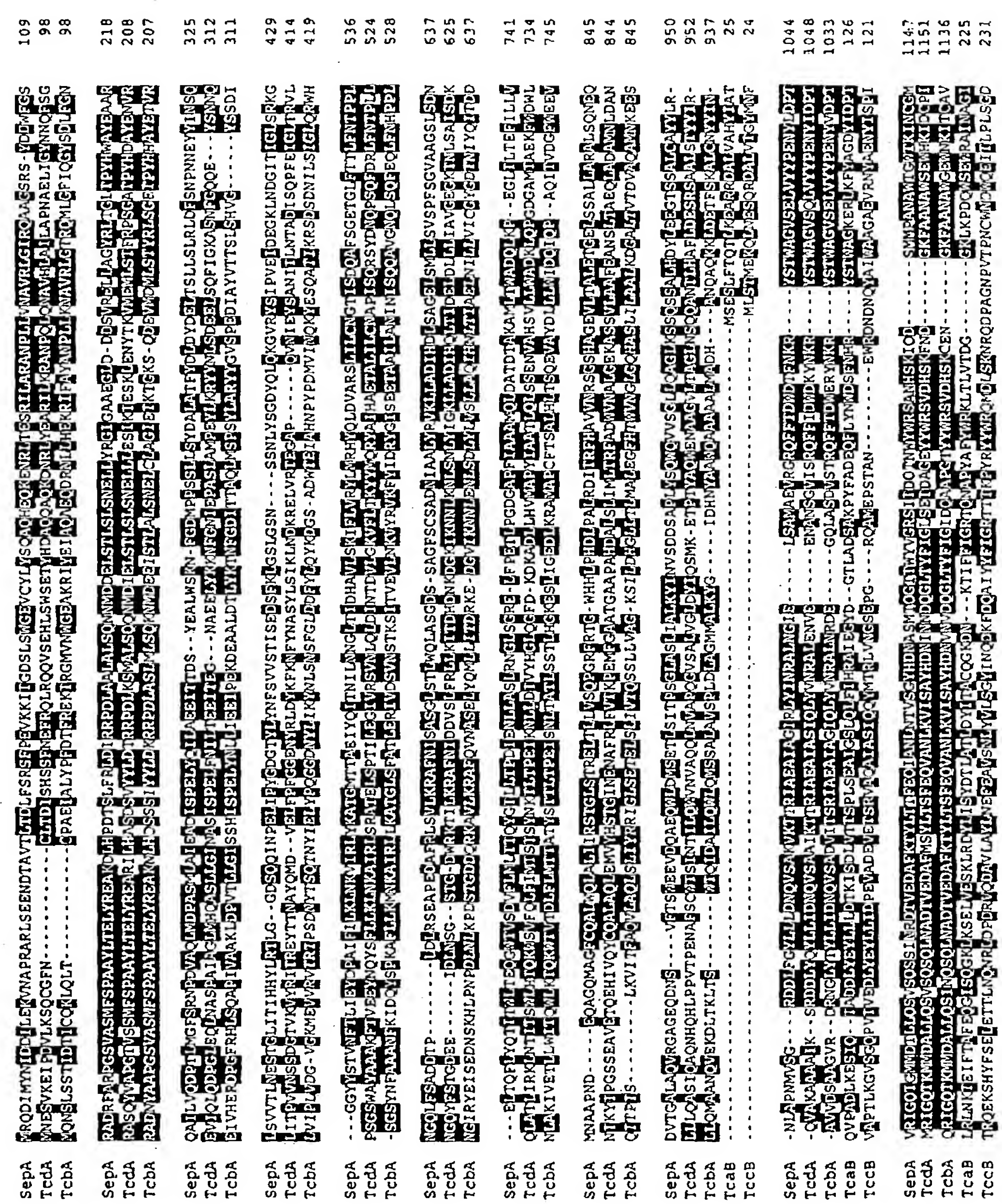

FIGURE 4 – continued

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SepA  NPWSDLVCSVFFNSRLYVVWVEEN----QSADTEAESTTTTQQSYTLKLSFRRYDGTWSSPVSFDIIGNIAFPETQGMHVTCNPLTEQLYCAFYS--VISKPDFDNAQLI  1251
TcdA  NPYKSTIRPVIYKSRLYLLWLEQKEITKQTGNSKDGYQTETDYRYELKLAHIRYDGTWNTPITFDVNKKISELKLEKNRAP------GLYCAGYQGQDTLLVMFYNQQDT  1255
TcbA  NPWKNIIRPVVYMSRLYLLWLEQ-----QSKKSDD--GKTTIYQYNLKLAHIRYDGSWNTPFTFDVTEKVKNYTSSTDAAES----LGLYCTGYQGEDTLLVMFYSMQSS  1235
TcaB  -------------SEAYSGHVEPFWE----------------NNK----LHIRWFTISKEDKIDFVYKNIWVMSSDYSWASK-------------------------------  274
TccB  TVLEHTVRPVFYNDRLYVAWVERD---PAVQKDADGKNIGKTHAYNIKFGYKRYDDTWTAPNTTTLMTQQAGESSETQRSSLLID----ESSTTLRQVNLLATTDFSIDP  334

SepA  SVDNDMTLNVISDIGIFKSVSHEFNI-----------------------STEKFINNVFSDPS-----ANYFVSATSLIDDVIHSDFSLLNSKTTSTVFTNEDSSLLTPEL  1334
TcdA  LDSYKN--ASMQGLYIFADMASKDMTPEQ-------------SNVYRDNSYQQFDTNNV------------RRVNNRYAEDYEIPSSVSSR------------KDYGWGDY  1327
TcbA  YSSYTDNNAPVTGLYIFADMSSDNMTNAQ-------------ATNYWNNSYPQFDTVMADPDSDNKKVITRRVNNRYAEDYEIPSSVTSN------------SNYSWGDH  1320
TcaB  ----KK-----ILELSFTDYNRVGATG-----------------------------SSS---------------------------------------------------  295
TccB  TEETDSNPYGRLMLGVFVRQFEGDGANRKNKPVVYGYLYCDSAFNRHVLRPLSKNFLFSTYRDETDGQNSLQFAVYDKKYVITKVVTGATEDPE---------NTGWVSK  435

SepA  HIIANVSCFVSTAGIATQSTIEKFVQAGIEFEEINFYAG----------QAAGGFDGFVGVDV-SNSKVYQVGKEAVGVTVKSYSVTGVSGSVELFIDSS----------  1423
TcdA  YLSMVYNGDIPTINYKAASSDLKIYISPKLRIIHNGYEGQKRNQCNLMNKYGKLGDKFIVYTS-LGVNPNNSSNKLMFYPVYQYSGN-TSGLNQGRLLFHRDTTYPSKVE  1435
TcbA  SLTMLYGGSVPNITFESAAEDLRLSTNMALSIIHNGYAGTRRIQCNLMKQYASLGDKFIIYDS-SFDDANRFNLVPLFKFGKDENSD-DSICIYNENPSSEDKKWYFSSK  1428
TcaB  ----------PTEVASQYGSDAQMNISDDGTVLIFQNAG----------GATPSTGVTLCYDS-GNVIKNLSSTGSANLSSKDYATI-----------------------  361
TccB  VDDLKQGTTGAYVYIDQDGLTLHIQTTTNGDFINRHTFG---------------YNDLVYDSKSGYGFTWSGNEGFYLDYHDGNYYTFHNAIINYYPSGYGGGS-VPNG  528

SepA  --NKYFSGILSDKMITALISGSTSKVN---YVSSIGSQD--FWSVKSLMPALQIYELIDDII-----------LTSGVNGTEIKSWPSAEWYNDKLSLQS-----GNNLF  1510
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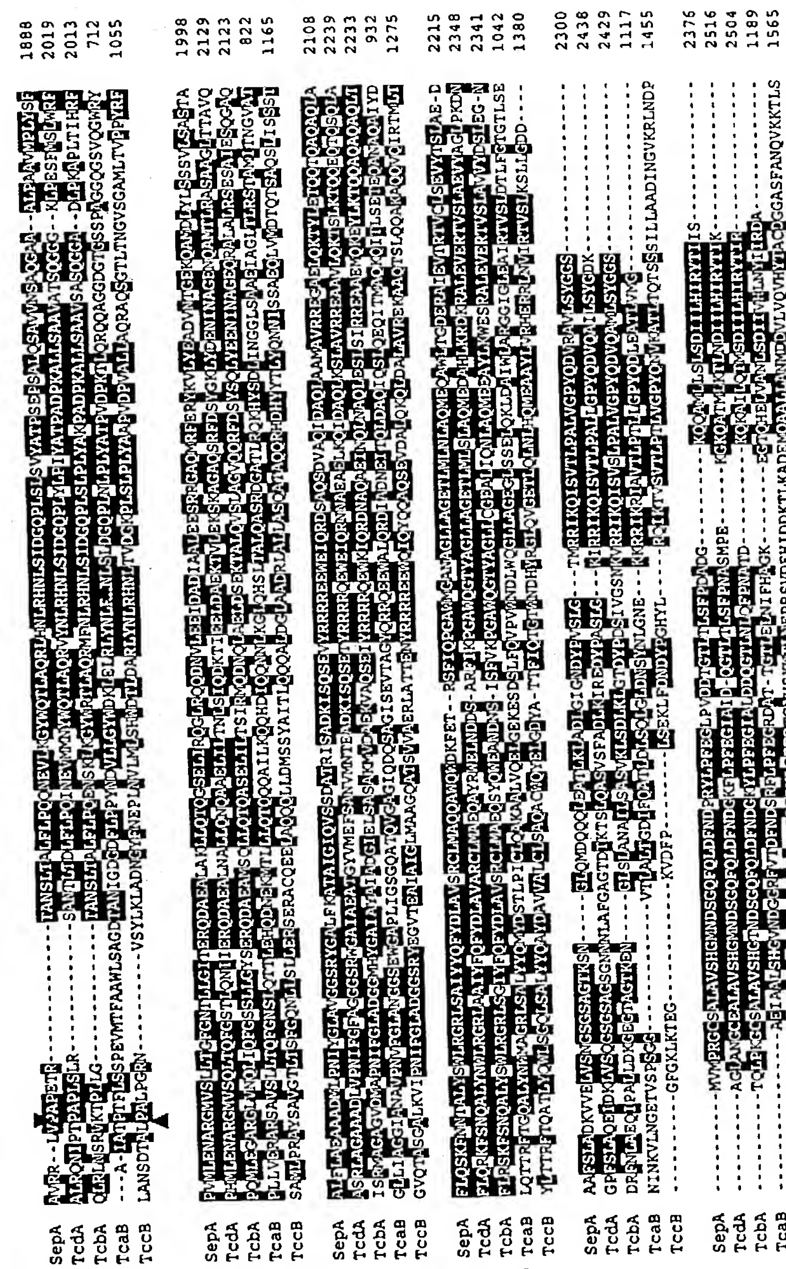

FIGURE 4 - continued

FIGURE 5

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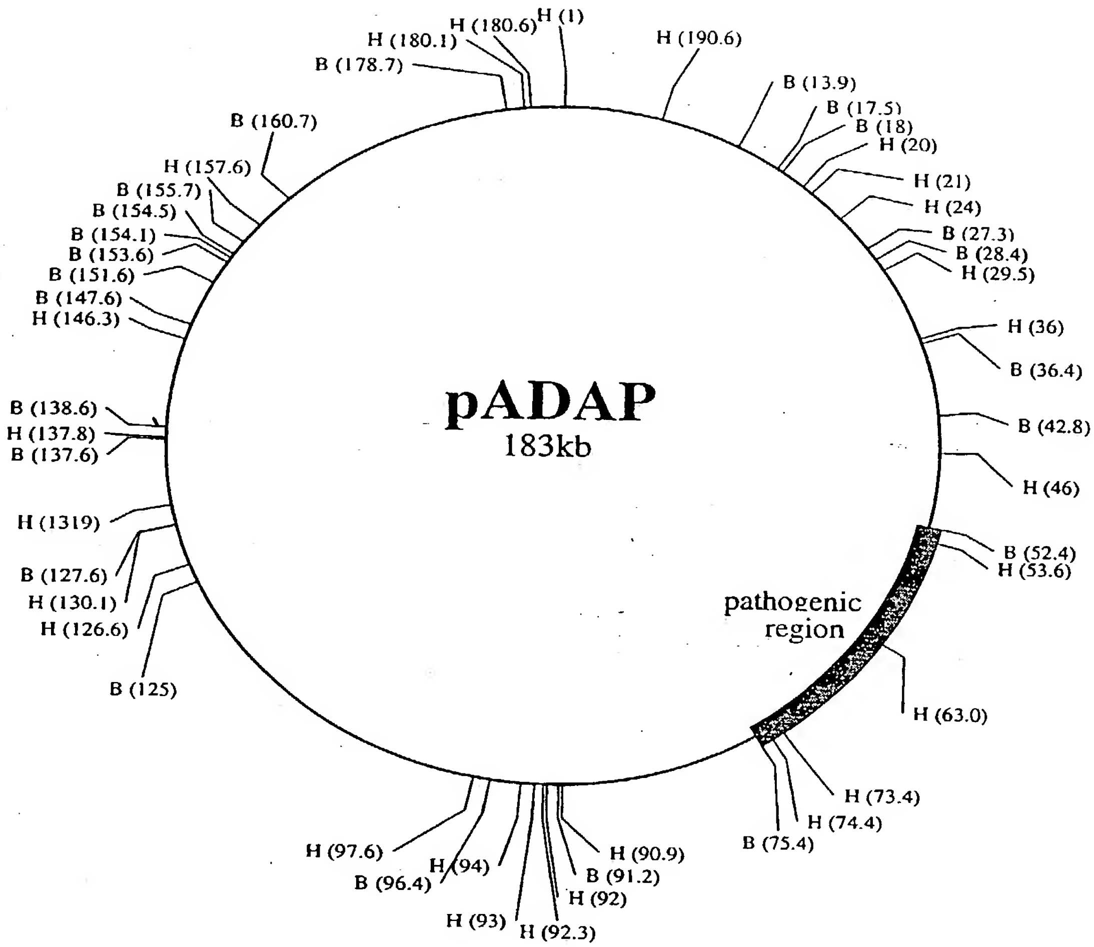
FIGURE 6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Atty Docket No. 24747-1104US

DECLARATION FOR PATENT APPLICATION

As a below-named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled:

NUCLEOTIDE SEQUENCES ENCODING AN INSECTICIDAL PROTEIN COMPLEX FROM SERRATIA

the specification of which

( ) is attached hereto.
( ) was filed by an authorized person on my behalf on ___________ as Application Serial No.___________
(X) was filed as PCT Application Serial No. PCT/NZ00/00174 on 04 September, 2000.
(X) amended in a Preliminary Amendment filed March 1, 2002.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, §119(a)-(d) or §365(b) of any foreign application(s) for patent or inventor's certificate listed below and so identified, or §365(a) of any PCT international application that designated at least one country other than the United States of America, listed below, and I have also identified below any foreign application for patent or inventor's certificate or PCT international application on this invention filed by us or our legal representatives or assigns and having a filing date before that of the application on which priority is claimed.

Number	Country	Day/Month/Year Filed	Priority Claimed (Yes or No)
337610	NEW ZEALAND	02 September 1999	Yes

I hereby claim benefit under Title 35, United States Code, §119(e) of any United States provisional application(s) listed below:

Application Serial No.	Filing Date
N/A	

Atty Docket No. 24747-1104US

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

Application Serial No.	Filing Date	Status
N/A		

PCT Application No.	Filing Date	Status
N/A		

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

I hereby appoint the following attorneys and agents, with full power of substitution and revocation, to prosecute this application and to transact all business in the United States Patent and Trademark Office connected therewith and request that all correspondence and telephone calls in respect to this application be directed to Stephanie Seidman, HELLER EHRMAN WHITE AND McAULIFFE LLP, 4350 La Jolla Village Drive, 7th Floor, San Diego, California 92122-1246; 858-450-8400:

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(4)

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Atty Docket No. 24747-1104US

1-00

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2-00

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Citizenship:	New Zealand

3-00

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Citizenship:	New Zealand

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Ser Thr Gly Leu Ile Thr Ile His His Tyr Leu Arg Thr Leu Gly Gly
        480                 485                 490

gac tca cag cag att aac cct gag ctt ata cct tat ggg gat gga aca      3508
Asp Ser Gln Gln Ile Asn Pro Glu Leu Ile Pro Tyr Gly Asp Gly Thr
    495                 500                 505

tat ctt tat aat ttc agc gtg gtg tca acg ata tca gag gat agt ttc      3556
Tyr Leu Tyr Asn Phe Ser Val Val Ser Thr Ile Ser Glu Asp Ser Phe
510                 515                 520                 525

aaa cta ggg tcg tta ggt tct aac agt agc aat ctt tac tct ggg gat      3604
Lys Leu Gly Ser Leu Gly Ser Asn Ser Ser Asn Leu Tyr Ser Gly Asp
                530                 535                 540

tat cag ctt caa aaa ggg gtt cgc tat agc att cct gtt gaa ata gat      3652
Tyr Gln Leu Gln Lys Gly Val Arg Tyr Ser Ile Pro Val Glu Ile Asp
            545                 550                 555

gaa gga aag tta aat gat ggg atc aca ata gga ttg agt agg aaa ggg      3700
Glu Gly Lys Leu Asn Asp Gly Ile Thr Ile Gly Leu Ser Arg Lys Gly
        560                 565                 570

ggg gga tat tac tca aca gta aac ttc act ctg att gaa tat gat cct      3748
Gly Gly Tyr Tyr Ser Thr Val Asn Phe Thr Leu Ile Glu Tyr Asp Pro
    575                 580                 585

gcg ata ttc att ctt aaa tta aat aaa gtt atc cgc cta tac aag gcc      3796
Ala Ile Phe Ile Leu Lys Leu Asn Lys Val Ile Arg Leu Tyr Lys Ala
590                 595                 600                 605

acg ggc atg acc acg gcg gaa ata tat caa atc acc aat att ctt aat      3844
Thr Gly Met Thr Thr Ala Glu Ile Tyr Gln Ile Thr Asn Ile Leu Asn
                610                 615                 620

aac ggt ctc acc att gac cat gcg gtc ctg agt aaa atc ttc ctg gtc      3892
Asn Gly Leu Thr Ile Asp His Ala Val Leu Ser Lys Ile Phe Leu Val
            625                 630                 635

cgt tac ctg atg cgt cac tat cag ctt gat gtg gcc cgg tca ctg ata      3940
Arg Tyr Leu Met Arg His Tyr Gln Leu Asp Val Ala Arg Ser Leu Ile
        640                 645                 650

ttg tgc aac gga acc atc agt gac cag gcg ttc agc ggc gaa acc ggc      3988
Leu Cys Asn Gly Thr Ile Ser Asp Gln Ala Phe Ser Gly Glu Thr Gly
    655                 660                 665

ctg ttc acc acg ctg ttc aac acc cca ccg ctg aac ggc cag ctg ttt      4036
Leu Phe Thr Thr Leu Phe Asn Thr Pro Pro Leu Asn Gly Gln Leu Phe
670                 675                 680                 685

tct gca gat gat acc ccc ctc gac tta cgc tct gaa gca ccg gag gat      4084
Ser Ala Asp Asp Thr Pro Leu Asp Leu Arg Ser Glu Ala Pro Glu Asp
                690                 695                 700
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gct ttc cgt ctc agc gta ctg aaa cgc gca ttt aac atc agc gcc tcg     4132
Ala Phe Arg Leu Ser Val Leu Lys Arg Ala Phe Asn Ile Ser Ala Ser
            705                     710                     715

ggg ctt tcc acg ctc tgg cag ttg gcc agc ggt gac agc agc gct ggg     4180
Gly Leu Ser Thr Leu Trp Gln Leu Ala Ser Gly Asp Ser Ser Ala Gly
        720                     725                     730

ttt agc tgc tct gct gac aat atc gcc gca ctc tac cga gtg aaa ctc     4228
Phe Ser Cys Ser Ala Asp Asn Ile Ala Ala Leu Tyr Arg Val Lys Leu
    735                     740                     745

ctg gct gac atc cac gac cta tcc gct ggt gag ctg tca atg ttg ctg     4276
Leu Ala Asp Ile His Asp Leu Ser Ala Gly Glu Leu Ser Met Leu Leu
750                     755                     760                 765

tcc gtc tcc cct ttc agc ggg gtg gcc gcc ggc tcg ctg tcc gat aat     4324
Ser Val Ser Pro Phe Ser Gly Val Ala Ala Gly Ser Leu Ser Asp Asn
                770                     775                     780

gag ctg acg cag ttt ctg tac cag acc acc acc tgg ctc acg gag cag     4372
Glu Leu Thr Gln Phe Leu Tyr Gln Thr Thr Thr Trp Leu Thr Glu Gln
            785                     790                     795

ggc tgg acg gtc agc gat gtg ttc ctg atg ctg acg acg cag tac ggt     4420
Gly Trp Thr Val Ser Asp Val Phe Leu Met Leu Thr Thr Gln Tyr Gly
        800                     805                     810

acc ctg ctg acc ccc gac att gag aac ctg ctc gct tcc ctg cgc aac     4468
Thr Leu Leu Thr Pro Asp Ile Glu Asn Leu Leu Ala Ser Leu Arg Asn
    815                     820                     825

gga ctg tcg ggc cgt gag ctg ttc ccg gaa acg ctc ccc ggc gat ggc     4516
Gly Leu Ser Gly Arg Glu Leu Phe Pro Glu Thr Leu Pro Gly Asp Gly
830                     835                     840                 845

gct ccc ttt att gcc gcc gcc atg cag ctg gac gcc acg gat acg gcg     4564
Ala Pro Phe Ile Ala Ala Ala Met Gln Leu Asp Ala Thr Asp Thr Ala
                850                     855                     860

aag gcg atg ctg act tgg gcg gac cag ttg aag cca gag ggg ctg acg     4612
Lys Ala Met Leu Thr Trp Ala Asp Gln Leu Lys Pro Glu Gly Leu Thr
            865                     870                     875

ctg acg gaa ttt att ctt ttg gtg atg aat gcc gcc cca aat gac gag     4660
Leu Thr Glu Phe Ile Leu Leu Val Met Asn Ala Ala Pro Asn Asp Glu
        880                     885                     890

cag gcg ggc cag atg gca ggg ttc tgc caa gcc ctg tgg caa ctg gca     4708
Gln Ala Gly Gln Met Ala Gly Phe Cys Gln Ala Leu Trp Gln Leu Ala
    895                     900                     905

ctg atc atc cgc agc acc ggc ctc agc acg cgc gag ctg acg ctg ctg     4756
Leu Ile Ile Arg Ser Thr Gly Leu Ser Thr Arg Glu Leu Thr Leu Leu
910                     915                     920                 925

gtc agc cag ccg gga cgc ttc cgc aca gga tgg cac cat ctg ccc cat     4804
Val Ser Gln Pro Gly Arg Phe Arg Thr Gly Trp His His Leu Pro His
                930                     935                     940

gac ctg ccg gcg ctt cgc gac att acg cgt ttt cat gcc gtc gtt aac     4852
Asp Leu Pro Ala Leu Arg Asp Ile Thr Arg Phe His Ala Val Val Asn
            945                     950                     955

cgc agc ggc agc cat gcc ggg gag gtc ctg acc gca ctt gag acc gga     4900
Arg Ser Gly Ser His Ala Gly Glu Val Leu Thr Ala Leu Glu Thr Gly
        960                     965                     970
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gaa ctg tcg tca gcc ctg ctg gcc cgg gcc ctg tca cag aat gag cag 4948
Glu Leu Ser Ser Ala Leu Leu Ala Arg Ala Leu Ser Gln Asn Glu Gln
975 980 985

gat gtg acc ggc gcc ttg gcg cag gtg agg ggg gcc ggt gaa cag gac 4996
Asp Val Thr Gly Ala Leu Ala Gln Val Arg Gly Ala Gly Glu Gln Asp
990 995 1000 1005

aac agc gtg ttc acc tcc tgg gaa gag gtg gac cag gct gag cag tgg 5044
Asn Ser Val Phe Thr Ser Trp Glu Glu Val Asp Gln Ala Glu Gln Trp
1010 1015 1020

ctg gac atg agt gag acc ctg tcc att acg cca tcc ggt ctg gct agc 5092
Leu Asp Met Ser Glu Thr Leu Ser Ile Thr Pro Ser Gly Leu Ala Ser
1025 1030 1035

ctg att gcc ctg aag tac atc aat gtg tcc gat gac agt gca ccg ttg 5140
Leu Ile Ala Leu Lys Tyr Ile Asn Val Ser Asp Asp Ser Ala Pro Leu
1040 1045 1050

tac agc cag tgg cag gtg gta tcc ggt ctg ctg cag gcc ggg ctg aaa 5188
Tyr Ser Gln Trp Gln Val Val Ser Gly Leu Leu Gln Ala Gly Leu Lys
1055 1060 1065

agc agc cag agc tcg gcg ctg cac gat tat ctg gag gag ggg acc agc 5236
Ser Ser Gln Ser Ser Ala Leu His Asp Tyr Leu Glu Glu Gly Thr Ser
1070 1075 1080 1085

agc gcc ctt tgt gcg tat tat ctg cgt aat ctg gca ccg aac atg gta 5284
Ser Ala Leu Cys Ala Tyr Tyr Leu Arg Asn Leu Ala Pro Asn Met Val
1090 1095 1100

tcc ggg cgc gat gac ctc ttc ggg tat ctg ctg ctg gat aat cag gtg 5332
Ser Gly Arg Asp Asp Leu Phe Gly Tyr Leu Leu Leu Asp Asn Gln Val
1105 1110 1115

tca gcc aag gta aaa acc acc cgc att gcg gag gcc atc gcc ggc ata 5380
Ser Ala Lys Val Lys Thr Thr Arg Ile Ala Glu Ala Ile Ala Gly Ile
1120 1125 1130

cgg ctg tat atc aac cgg gcc ctt aac gga ata gaa ctc agc gcc atg 5428
Arg Leu Tyr Ile Asn Arg Ala Leu Asn Gly Ile Glu Leu Ser Ala Met
1135 1140 1145

gca gag gtg agg ggg cgt cag ttt ttc act gac tgg gat acg ttc aac 5476
Ala Glu Val Arg Gly Arg Gln Phe Phe Thr Asp Trp Asp Thr Phe Asn
1150 1155 1160 1165

aaa cgt tac agc acc tgg gcg ggc gtc tca gag ctg gtt tac tat ccg 5524
Lys Arg Tyr Ser Thr Trp Ala Gly Val Ser Glu Leu Val Tyr Tyr Pro
1170 1175 1180

gaa aac tac ctc gac ccg acg gtc cgt atc ggg cag acc ggc atg atg 5572
Glu Asn Tyr Leu Asp Pro Thr Val Arg Ile Gly Gln Thr Gly Met Met
1185 1190 1195

gac acc ctg ctg cag tct gtc agc cag agc agt atc aac cgc gat acc 5620
Asp Thr Leu Leu Gln Ser Val Ser Gln Ser Ser Ile Asn Arg Asp Thr
1200 1205 1210

gtg gag gat gcc ttt aaa acc tat ctg acc acg ttt gag cag att gcc 5668
Val Glu Asp Ala Phe Lys Thr Tyr Leu Thr Thr Phe Glu Gln Ile Ala
1215 1220 1225

aat ctg aac act gtc agc gga tat cac gat aac gcc agc atg acg cag 5716
Asn Leu Asn Thr Val Ser Gly Tyr His Asp Asn Ala Ser Met Thr Gln

1230 1235 1240 1245

ggg act aca tgg tat gtg ggt cgc agc atc aca gat cag act aac tgg 5764
Gly Thr Thr Trp Tyr Val Gly Arg Ser Ile Thr Asp Gln Thr Asn Trp
1250 1255 1260

tac tgg cgc agc gcc aac cac agc aaa atc caa gac tca atg atg ccc 5812
Tyr Trp Arg Ser Ala Asn His Ser Lys Ile Gln Asp Ser Met Met Pro
1265 1270 1275

gcg aat gcc tgg acc gga tgg aca aaa att aac tgc gga atg aat ccg 5860
Ala Asn Ala Trp Thr Gly Trp Thr Lys Ile Asn Cys Gly Met Asn Pro
1280 1285 1290

tgg tca gat ctt gtg tgc tcg gtg ttt ttc aac agt cgc ctt tat gtc 5908
Trp Ser Asp Leu Val Cys Ser Val Phe Phe Asn Ser Arg Leu Tyr Val
1295 1300 1305

gtc tgg gtc gaa gag aat cag tct gct gat acg gag gca gag agc acg 5956
Val Trp Val Glu Glu Asn Gln Ser Ala Asp Thr Glu Ala Glu Ser Thr
1310 1315 1320 1325

aca acc acg cag cag agc tac acg ctg aaa ctg tcg ttc cgg cgc tac 6004
Thr Thr Thr Gln Gln Ser Tyr Thr Leu Lys Leu Ser Phe Arg Arg Tyr
1330 1335 1340

gac ggt aca tgg agt tcc ccg gtg tcg ttc gac att acc ggc aac atc 6052
Asp Gly Thr Trp Ser Ser Pro Val Ser Phe Asp Ile Thr Gly Asn Ile
1345 1350 1355

gca ttt ccg gaa acg cag ggc atg cat gtg acc tgt aat ccc ctg act 6100
Ala Phe Pro Glu Thr Gln Gly Met His Val Thr Cys Asn Pro Leu Thr
1360 1365 1370

gag cag ctc tat tgc gcg ttt tac tcc gtc acc agc aag ccg gac ttt 6148
Glu Gln Leu Tyr Cys Ala Phe Tyr Ser Val Thr Ser Lys Pro Asp Phe
1375 1380 1385

gat aac gct cag ctg att tct gtg gat aat gat atg acg cta aat gtc 6196
Asp Asn Ala Gln Leu Ile Ser Val Asp Asn Asp Met Thr Leu Asn Val
1390 1395 1400 1405

atc tca gat ata ggg att ttt aag agc gtc agt cac gaa ttt aat acg 6244
Ile Ser Asp Ile Gly Ile Phe Lys Ser Val Ser His Glu Phe Asn Thr
1410 1415 1420

agc act gag aaa ttt att aat aat gtt ttt tca gac cct tcc gct aat 6292
Ser Thr Glu Lys Phe Ile Asn Asn Val Phe Ser Asp Pro Ser Ala Asn
1425 1430 1435

tat ttt gtc agt gca acg agt tta att gat gat gtt atc cac agc gat 6340
Tyr Phe Val Ser Ala Thr Ser Leu Ile Asp Asp Val Ile His Ser Asp
1440 1445 1450

ttc tca ctc ctt aat tct aaa act aca agt act gtt ttt act aat gaa 6388
Phe Ser Leu Leu Asn Ser Lys Thr Thr Ser Thr Val Phe Thr Asn Glu
1455 1460 1465

gat tcc tct ctt ttg acg cca gag ctt cat att aca gca aat gtt tcg 6436
Asp Ser Ser Leu Leu Thr Pro Glu Leu His Ile Thr Ala Asn Val Ser
1470 1475 1480 1485

tgt ttt gtt agt act gct ggc atc gcc act caa tct acc ata gaa aaa 6484
Cys Phe Val Ser Thr Ala Gly Ile Ala Thr Gln Ser Thr Ile Glu Lys
1490 1495 1500

ttc gtt cag gca ggg ata gaa ttt gag gaa att aat ttt tat gca ggc 6532

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Phe Val Gln Ala Gly Ile Glu Phe Glu Glu Ile Asn Phe Tyr Ala Gly
            1505                1510                1515

cag gcc gcc ggc gga ttt gac gga ttt gtg gga gtg gat gtt tct aat      6580
Gln Ala Ala Gly Gly Phe Asp Gly Phe Val Gly Val Asp Val Ser Asn
        1520                1525                1530

tca aaa gta tac cag gtc gga aaa gaa gca gtt ggt gtc act gta aaa      6628
Ser Lys Val Tyr Gln Val Gly Lys Glu Ala Val Gly Val Thr Val Lys
    1535                1540                1545

tct tat tcc gtc act ggc gtt agt ggt tct gtt gag tta ttt att gat      6676
Ser Tyr Ser Val Thr Gly Val Ser Gly Ser Val Glu Leu Phe Ile Asp
1550                1555                1560                1565

tca tca aat aaa tac ttc agc gga att ttg tca gat aaa atg ata acc      6724
Ser Ser Asn Lys Tyr Phe Ser Gly Ile Leu Ser Asp Lys Met Ile Thr
                1570                1575                1580

gct tta att agc ggc agt aca tca aaa gtt aat tac gtg tcg tct att      6772
Ala Leu Ile Ser Gly Ser Thr Ser Lys Val Asn Tyr Val Ser Ser Ile
            1585                1590                1595

ggc tct caa gat ttt tgg agt gta aag tcg ctc atg ccg gca ctt cag      6820
Gly Ser Gln Asp Phe Trp Ser Val Lys Ser Leu Met Pro Ala Leu Gln
        1600                1605                1610

ata tat gaa tta atc gat gat atc ata ctg aca tcc ggc gta aat ggg      6868
Ile Tyr Glu Leu Ile Asp Asp Ile Ile Leu Thr Ser Gly Val Asn Gly
    1615                1620                1625

act gaa att aaa tcc tgg cct tcc gct gaa tgg tat aat gat aag ctg      6916
Thr Glu Ile Lys Ser Trp Pro Ser Ala Glu Trp Tyr Asn Asp Lys Leu
1630                1635                1640                1645

agt ctg caa tcc ggg aat aat ctt ttc aac acc aaa tcg ctg agt ttt      6964
Ser Leu Gln Ser Gly Asn Asn Leu Phe Asn Thr Lys Ser Leu Ser Phe
                1650                1655                1660

acc gtt aat acc agt gat att gtt gaa gat gag ttt gac gtg acg ttt      7012
Thr Val Asn Thr Ser Asp Ile Val Glu Asp Glu Phe Asp Val Thr Phe
            1665                1670                1675

acg ttc acc gct gtc gat cag aat aac gtc gtg ctg gcc gcc cgg acg      7060
Thr Phe Thr Ala Val Asp Gln Asn Asn Val Val Leu Ala Ala Arg Thr
        1680                1685                1690

gcc ata tta acc gtc att cga aac att aat aat gac act tcc gtt atc      7108
Ala Ile Leu Thr Val Ile Arg Asn Ile Asn Asn Asp Thr Ser Val Ile
    1695                1700                1705

gca tta cgt aaa aat acg cgt ggc gcg cag tat att cgt ttc act gcg      7156
Ala Leu Arg Lys Asn Thr Arg Gly Ala Gln Tyr Ile Arg Phe Thr Ala
1710                1715                1720                1725

ggt aac gat gtg gcg ctt att cgc ctc aac acc ctc ttt gcc cgc caa      7204
Gly Asn Asp Val Ala Leu Ile Arg Leu Asn Thr Leu Phe Ala Arg Gln
                1730                1735                1740

ctg gtc gac cgg gcg aat acc ggg att gac acc att ctt tcc atg gag      7252
Leu Val Asp Arg Ala Asn Thr Gly Ile Asp Thr Ile Leu Ser Met Glu
            1745                1750                1755

acc cag agg ctt acc gaa ccc gcc ctg gaa gag ggg agt gat gtg ttt      7300
Thr Gln Arg Leu Thr Glu Pro Ala Leu Glu Glu Gly Ser Asp Val Phe
        1760                1765                1770
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atg gac ttc tcc gga gcc aat gcc ctc tat ttc tgg gag ctg ttc tat      7348
Met Asp Phe Ser Gly Ala Asn Ala Leu Tyr Phe Trp Glu Leu Phe Tyr
    1775                1780                1785

tac acg ccg atg atg gtg ttc cag cgg ttg ttg cag gaa cag cac ttc      7396
Tyr Thr Pro Met Met Val Phe Gln Arg Leu Leu Gln Glu Gln His Phe
1790                1795                1800                1805

ccg gaa gcc acc cgc tgg ctg cag tat gtc tgg aac ccg gcc ggg cac      7444
Pro Glu Ala Thr Arg Trp Leu Gln Tyr Val Trp Asn Pro Ala Gly His
                1810                1815                1820

gtg gta aac ggg gtg ctg cag aat tac acc tgg aat gtc cgt ccg ctg      7492
Val Val Asn Gly Val Leu Gln Asn Tyr Thr Trp Asn Val Arg Pro Leu
            1825                1830                1835

gag gag gac acc ggc tgg aac gac tcg ccg ctg gac tcc att gac ccc      7540
Glu Glu Asp Thr Gly Trp Asn Asp Ser Pro Leu Asp Ser Ile Asp Pro
        1840                1845                1850

gat gca ata gcc cag tac gac ccc atg cat tac aag gtc gcc acc ttt      7588
Asp Ala Ile Ala Gln Tyr Asp Pro Met His Tyr Lys Val Ala Thr Phe
    1855                1860                1865

atg tcg tac ctc gac ctg ctg att gcc cgc ggt gat gcc gcc tac cgg      7636
Met Ser Tyr Leu Asp Leu Leu Ile Ala Arg Gly Asp Ala Ala Tyr Arg
1870                1875                1880                1885

ctg ctc gag cgg gac acc ctt aac gag gcc cgg atg tgg tac gtc cag      7684
Leu Leu Glu Arg Asp Thr Leu Asn Glu Ala Arg Met Trp Tyr Val Gln
                1890                1895                1900

gcc ctg aac ctt ctg ggc gac gag ccc tat att tcc ttt gac gcc gac      7732
Ala Leu Asn Leu Leu Gly Asp Glu Pro Tyr Ile Ser Phe Asp Ala Asp
            1905                1910                1915

tgg tcg gcg ttg acc ctg ggt gac gca gcc agc gag gtg acg cga cgc      7780
Trp Ser Ala Leu Thr Leu Gly Asp Ala Ala Ser Glu Val Thr Arg Arg
        1920                1925                1930

gat tac cag gag gcc ctg ctg gcc gtg cgc cgg ttg gtg ccc gct ccc      7828
Asp Tyr Gln Glu Ala Leu Leu Ala Val Arg Arg Leu Val Pro Ala Pro
    1935                1940                1945

gag aca cgg acg gcg aat tcc ctg acg gca ctg ttc ctc ccg cag cag      7876
Glu Thr Arg Thr Ala Asn Ser Leu Thr Ala Leu Phe Leu Pro Gln Gln
1950                1955                1960                1965

aac gag gtg ctc aaa ggc tac tgg caa acc ttg gca cag cgg ctc cat      7924
Asn Glu Val Leu Lys Gly Tyr Trp Gln Thr Leu Ala Gln Arg Leu His
                1970                1975                1980

aac ctg cgc cac aac ctc tcc att gac ggc cag ccg ctt tcc ctg tcc      7972
Asn Leu Arg His Asn Leu Ser Ile Asp Gly Gln Pro Leu Ser Leu Ser
            1985                1990                1995

gtc tac gcc acg ccg tcc gaa ccg tcc gcc ctg cag agt gcc gtc gtc      8020
Val Tyr Ala Thr Pro Ser Glu Pro Ser Ala Leu Gln Ser Ala Val Val
        2000                2005                2010

aac agc gcg cag ggt gct gca gca ctg ccg gcc gcg gtg atg ccg ctt      8068
Asn Ser Ala Gln Gly Ala Ala Ala Leu Pro Ala Ala Val Met Pro Leu
    2015                2020                2025

tac agt ttc ccg gtc atg ctg gag aac gcc cgg ggg atg gtg agc ctg      8116
Tyr Ser Phe Pro Val Met Leu Glu Asn Ala Arg Gly Met Val Ser Leu
2030                2035                2040                2045
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ctg acc ggg ttc ggc aac aca ctg ctc ggt att acc gag cgt cag gat 8164
Leu Thr Gly Phe Gly Asn Thr Leu Leu Gly Ile Thr Glu Arg Gln Asp
2050 2055 2060

gcg gag gcg ctg gcc aaa ctg ctg cag acc cag ggc agt gaa ctg ata 8212
Ala Glu Ala Leu Ala Lys Leu Leu Gln Thr Gln Gly Ser Glu Leu Ile
2065 2070 2075

cgc cag ggc ctt cgc cag cag gat aac gtc ctc gag gaa atc gat gcg 8260
Arg Gln Gly Leu Arg Gln Gln Asp Asn Val Leu Glu Glu Ile Asp Ala
2080 2085 2090

gat att gcc gcc ctg gag gag agc cgc cgc ggc gcg cag atg cgt ttt 8308
Asp Ile Ala Ala Leu Glu Glu Ser Arg Arg Gly Ala Gln Met Arg Phe
2095 2100 2105

gaa cgt tac aaa gtg ttg tac gag gcg gac gtc aac acc ggc gaa aaa 8356
Glu Arg Tyr Lys Val Leu Tyr Glu Ala Asp Val Asn Thr Gly Glu Lys
2110 2115 2120 2125

cag gcc atg gac ttg tac ctc agt tcg tcc gtg ctg tcg gca tca acc 8404
Gln Ala Met Asp Leu Tyr Leu Ser Ser Ser Val Leu Ser Ala Ser Thr
2130 2135 2140

gcc gcg ctc ttt ttg gcc gag gcc gcg gcc gat atg ctg ccc aat att 8452
Ala Ala Leu Phe Leu Ala Glu Ala Ala Ala Asp Met Leu Pro Asn Ile
2145 2150 2155

tac ggg ctg gcc gtc ggg ggc tcc cgc tat ggg gca cta ttt aaa gcc 8500
Tyr Gly Leu Ala Val Gly Gly Ser Arg Tyr Gly Ala Leu Phe Lys Ala
2160 2165 2170

acc gcc atc ggc atc cag gtg tcc tcc gat gcc acc cgc ata tca gcg 8548
Thr Ala Ile Gly Ile Gln Val Ser Ser Asp Ala Thr Arg Ile Ser Ala
2175 2180 2185

gac aaa atc agc cag tcg gaa gtg tac cgc cgt cgc cgg gag gag tgg 8596
Asp Lys Ile Ser Gln Ser Glu Val Tyr Arg Arg Arg Arg Glu Glu Trp
2190 2195 2200 2205

gaa atc cag cgt gat agt gcg cag tct gac gtg gcg cag att gat gcc 8644
Glu Ile Gln Arg Asp Ser Ala Gln Ser Asp Val Ala Gln Ile Asp Ala
2210 2215 2220

cag ctg gcg gcc atg gca gtg cgc cgg gaa ggg gct gag ctg cag aaa 8692
Gln Leu Ala Ala Met Ala Val Arg Arg Glu Gly Ala Glu Leu Gln Lys
2225 2230 2235

act tac ctt gag acc cag cag acc cag gca cag gcg cag ttg gca ttc 8740
Thr Tyr Leu Glu Thr Gln Gln Thr Gln Ala Gln Ala Gln Leu Ala Phe
2240 2245 2250

ctg cag agt aag ttc aac aat acg gct ctg tac agc tgg ctg cgg ggc 8788
Leu Gln Ser Lys Phe Asn Asn Thr Ala Leu Tyr Ser Trp Leu Arg Gly
2255 2260 2265

agg ttg tcc gcc att tat tac cag ttc tat gac ctg gca gta tcc cgc 8836
Arg Leu Ser Ala Ile Tyr Tyr Gln Phe Tyr Asp Leu Ala Val Ser Arg
2270 2275 2280 2285

tgc ctg atg gcg caa cag gcc tgg cag tgg gat aaa ttc gag act agg 8884
Cys Leu Met Ala Gln Gln Ala Trp Gln Trp Asp Lys Phe Glu Thr Arg
2290 2295 2300

tcg ttt atc cag ccg ggg gcc tgg atg ggg gca aat gcc ggt ctg ctg 8932
Ser Phe Ile Gln Pro Gly Ala Trp Met Gly Ala Asn Ala Gly Leu Leu

2305 2310 2315

gcc ggg gaa acc ctg atg ctg aat ctg gcg cag atg gag cag gcc tgg 8980
Ala Gly Glu Thr Leu Met Leu Asn Leu Ala Gln Met Glu Gln Ala Trp
2320 2325 2330

ctg acg ggg gat gag cgg gca ata gag gtg acg cgg acg gtc tgc ctg 9028
Leu Thr Gly Asp Glu Arg Ala Ile Glu Val Thr Arg Thr Val Cys Leu
2335 2340 2345

tcg gag gtc tat acc agc ctc gcg gag gat gcg gca ttc tct ctg gcc 9076
Ser Glu Val Tyr Thr Ser Leu Ala Glu Asp Ala Ala Phe Ser Leu Ala
2350 2355 2360 2365

gac aag gtg gtg gaa ctg gtc agt aac ggt tcg ggc agt gcg ggt acg 9124
Asp Lys Val Val Glu Leu Val Ser Asn Gly Ser Gly Ser Ala Gly Thr
2370 2375 2380

aaa agc aac gga tta cag atg gat caa cag caa ctc gag gcc acc ctg 9172
Lys Ser Asn Gly Leu Gln Met Asp Gln Gln Gln Leu Glu Ala Thr Leu
2385 2390 2395

aaa ctg gct gac ctc ggt atc ggc aac gat tac ccg gtc tcc ctt ggc 9220
Lys Leu Ala Asp Leu Gly Ile Gly Asn Asp Tyr Pro Val Ser Leu Gly
2400 2405 2410

acc atg agg cgc atc aaa caa ata agc gtc acg ctc ccg gcg ctg gtc 9268
Thr Met Arg Arg Ile Lys Gln Ile Ser Val Thr Leu Pro Ala Leu Val
2415 2420 2425

ggc ccc tat cag gac gtc cgt gcg gtt ctc agc tac ggc gga agt atg 9316
Gly Pro Tyr Gln Asp Val Arg Ala Val Leu Ser Tyr Gly Gly Ser Met
2430 2435 2440 2445

gtc atg ccc cgg ggt tgc agc gcg ctg gcg gtc tca cac gga atg aac 9364
Val Met Pro Arg Gly Cys Ser Ala Leu Ala Val Ser His Gly Met Asn
2450 2455 2460

gac agc ggc caa ttc caa ctg gat ttc aat gac ccg cgt tac ctg ccg 9412
Asp Ser Gly Gln Phe Gln Leu Asp Phe Asn Asp Pro Arg Tyr Leu Pro
2465 2470 2475

ttt gaa gga ctt cca gtt gat gac aca ggg acc ctg aca ctg agc ttc 9460
Phe Glu Gly Leu Pro Val Asp Asp Thr Gly Thr Leu Thr Leu Ser Phe
2480 2485 2490

ccg gat gct gac ggc aaa caa cag gcg atg ctc ctc agt ctg agc gac 9508
Pro Asp Ala Asp Gly Lys Gln Gln Ala Met Leu Leu Ser Leu Ser Asp
2495 2500 2505

atc atc ctg cat atc cgt tac acc att atc agc tga tag gtatcaacat 9557
Ile Ile Leu His Ile Arg Tyr Thr Ile Ile Ser * *
2510 2515 2520

agcgcaggcc cccgaacgag ggcctgcgag gagactgagc atg caa aat cat caa 9612
Met Gln Asn His Gln
2525

gac atg gcc att act gcc ccc acg ttg cct tcc ggg ggc ggt gcg gtc 9660
Asp Met Ala Ile Thr Ala Pro Thr Leu Pro Ser Gly Gly Gly Ala Val
2530 2535 2540

acc ggg ctc aag ggt gat atc gcg gcg gca ggg ccg gat ggt gcg gcg 9708
Thr Gly Leu Lys Gly Asp Ile Ala Ala Ala Gly Pro Asp Gly Ala Ala
2545 2550 2555

acc ctg agt att ccc ttg ccg gtt agc ccc ggt cgg ggt tac gcc ccc 9756

Thr Leu Ser Ile Pro Leu Pro Val Ser Pro Gly Arg Gly Tyr Ala Pro
2560 2565 2570

act ggg gca ctt aat tat cac agc cgg tcg ggg aac ggc ccc ttt ggc 9804
Thr Gly Ala Leu Asn Tyr His Ser Arg Ser Gly Asn Gly Pro Phe Gly
2575 2580 2585

att ggc tgg ggt atc ggc ggt gct gct gtc cag cgt cgt acg cgc aac 9852
Ile Gly Trp Gly Ile Gly Gly Ala Ala Val Gln Arg Arg Thr Arg Asn
2590 2595 2600 2605

gga gca cct acc tac gat gat act gat gaa ttc acc ggt ccg gac ggt 9900
Gly Ala Pro Thr Tyr Asp Asp Thr Asp Glu Phe Thr Gly Pro Asp Gly
2610 2615 2620

gag gtg ctg gtg ccg gca ctc acg gct gct ggc acc caa gaa gca cgg 9948
Glu Val Leu Val Pro Ala Leu Thr Ala Ala Gly Thr Gln Glu Ala Arg
2625 2630 2635

cag gcc acc tca cta ctg ggg ata aac cca ggc gga agc ttc aac gtt 9996
Gln Ala Thr Ser Leu Leu Gly Ile Asn Pro Gly Gly Ser Phe Asn Val
2640 2645 2650

cag gtt tac cgt tca cgt acg gag ggt agt ctc agc cgc ctt gag cgt 10044
Gln Val Tyr Arg Ser Arg Thr Glu Gly Ser Leu Ser Arg Leu Glu Arg
2655 2660 2665

tgg ctg ccc gcc gac gag aca gaa acg gaa ttt tgg gtg tta tat acc 10092
Trp Leu Pro Ala Asp Glu Thr Glu Thr Glu Phe Trp Val Leu Tyr Thr
2670 2675 2680 2685

cct gac gga cag gtg gct ctg ctg ggc cga aat gcg cag gct cgc atc 10140
Pro Asp Gly Gln Val Ala Leu Leu Gly Arg Asn Ala Gln Ala Arg Ile
2690 2695 2700

agc aac ccc aca gcc cca aca cag acg gcg gtt tgg ctg atg gag tcc 10188
Ser Asn Pro Thr Ala Pro Thr Gln Thr Ala Val Trp Leu Met Glu Ser
2705 2710 2715

tcg gta tca ctt acc ggc gaa cag atg tat tac caa tac cgt gcg gaa 10236
Ser Val Ser Leu Thr Gly Glu Gln Met Tyr Tyr Gln Tyr Arg Ala Glu
2720 2725 2730

gat gat gac ggt tgt gac gag gcg gag cgc gac gcg cac ccg cag gcc 10284
Asp Asp Asp Gly Cys Asp Glu Ala Glu Arg Asp Ala His Pro Gln Ala
2735 2740 2745

ggc gcc caa cgt tat ccg gtg gcg gtc tgg tat ggt aac cgt cag gcg 10332
Gly Ala Gln Arg Tyr Pro Val Ala Val Trp Tyr Gly Asn Arg Gln Ala
2750 2755 2760 2765

gct cgg acg cta ccg gcg ctg gtg tcg aca cca tca atg gat agc tgg 10380
Ala Arg Thr Leu Pro Ala Leu Val Ser Thr Pro Ser Met Asp Ser Trp
2770 2775 2780

ctg ttt atc ctg gtg ttt gat tat ggt gag cgt agc tcg gtg ctg tct 10428
Leu Phe Ile Leu Val Phe Asp Tyr Gly Glu Arg Ser Ser Val Leu Ser
2785 2790 2795

gaa gcg ccg gcc tgg caa aca cca gga agt ggg gag tgg ctg tgt cgt 10476
Glu Ala Pro Ala Trp Gln Thr Pro Gly Ser Gly Glu Trp Leu Cys Arg
2800 2805 2810

cag gat tgt ttt tcc ggg tat gag ttt ggt ttt aac ctg cgg act cgc 10524
Gln Asp Cys Phe Ser Gly Tyr Glu Phe Gly Phe Asn Leu Arg Thr Arg
2815 2820 2825

cgc ctg tgc cgt cag gtt ttg atg ttc cat tac cta ggt gtt ctg gcg 10572
Arg Leu Cys Arg Gln Val Leu Met Phe His Tyr Leu Gly Val Leu Ala
2830 2835 2840 2845

ggg agt tcg gga gcg aat gat gcg cca gca ttg att tct cgc ctg ttg 10620
Gly Ser Ser Gly Ala Asn Asp Ala Pro Ala Leu Ile Ser Arg Leu Leu
2850 2855 2860

ctg gac tac agg gaa agt cct tca ctc agt ctg ctc gag aac gtg cac 10668
Leu Asp Tyr Arg Glu Ser Pro Ser Leu Ser Leu Leu Glu Asn Val His
2865 2870 2875

cag gtg gct tat gag tcg gac ggg acg tct tgt gcc ttg ccg gca ctg 10716
Gln Val Ala Tyr Glu Ser Asp Gly Thr Ser Cys Ala Leu Pro Ala Leu
2880 2885 2890

gca ttg ggg tgg caa acc ttt acc ccg ccg aca ttg tcg gca tgg cag 10764
Ala Leu Gly Trp Gln Thr Phe Thr Pro Pro Thr Leu Ser Ala Trp Gln
2895 2900 2905

acg cgt gac gat atg ggc aag ttg agt ttg ctt caa ccc tat cag ctt 10812
Thr Arg Asp Asp Met Gly Lys Leu Ser Leu Leu Gln Pro Tyr Gln Leu
2910 2915 2920 2925

gta gac ctt aac ggc gaa ggt gtg gtg ggt atc ctg tat cag gac agc 10860
Val Asp Leu Asn Gly Glu Gly Val Val Gly Ile Leu Tyr Gln Asp Ser
2930 2935 2940

ggt gcc tgg tgg tac cgt gaa ccg gta cgc cag tcg ggg gat gat ccg 10908
Gly Ala Trp Trp Tyr Arg Glu Pro Val Arg Gln Ser Gly Asp Asp Pro
2945 2950 2955

gat gct gtg acc tgg ggg gcg gct gcg gcc ctg ccg aca atg ccc gct 10956
Asp Ala Val Thr Trp Gly Ala Ala Ala Ala Leu Pro Thr Met Pro Ala
2960 2965 2970

ttg cat aac agc ggc atc ctg gcg gat ctt aat ggg gat ggt cgg ctg 11004
Leu His Asn Ser Gly Ile Leu Ala Asp Leu Asn Gly Asp Gly Arg Leu
2975 2980 2985

gag tgg gtc gtt acc gcc ccc ggt gtg gcg ggg atg tat gat cgc acc 11052
Glu Trp Val Val Thr Ala Pro Gly Val Ala Gly Met Tyr Asp Arg Thr
2990 2995 3000 3005

ccc ggc cgc gac tgg ttg cat ttc acc ccc ctg tca gcc ttg ccc gta 11100
Pro Gly Arg Asp Trp Leu His Phe Thr Pro Leu Ser Ala Leu Pro Val
3010 3015 3020

gaa tat gcg cat cca aaa gca gtg ctc gcc gat atc ctg ggg gct ggg 11148
Glu Tyr Ala His Pro Lys Ala Val Leu Ala Asp Ile Leu Gly Ala Gly
3025 3030 3035

tta acg gac atg gtg ctt atc ggg ccg cgc agt gtt cgc ctc tat tcc 11196
Leu Thr Asp Met Val Leu Ile Gly Pro Arg Ser Val Arg Leu Tyr Ser
3040 3045 3050

ggc aaa aac gat ggt tgg aat aaa ggg gag acc gtg cag caa acg gaa 11244
Gly Lys Asn Asp Gly Trp Asn Lys Gly Glu Thr Val Gln Gln Thr Glu
3055 3060 3065

aga ctc act ctg ccg gtc ccg ggg gtt gac cca cgt acc ctc gtg gcg 11292
Arg Leu Thr Leu Pro Val Pro Gly Val Asp Pro Arg Thr Leu Val Ala
3070 3075 3080 3085

ttc agt gat atg gct ggc agt gga cag cag cat ttg acg gag gtg cgt 11340
Phe Ser Asp Met Ala Gly Ser Gly Gln Gln His Leu Thr Glu Val Arg
3090 3095 3100

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gct aat gga gta cgt tac tgg cca aac ctg ggg cac ggt cgt ttc ggt    11388
Ala Asn Gly Val Arg Tyr Trp Pro Asn Leu Gly His Gly Arg Phe Gly
            3105                3110                3115

cag ccg gtg aat att ccc ggt ttt agc cag tca gtg act acg ttt aac    11436
Gln Pro Val Asn Ile Pro Gly Phe Ser Gln Ser Val Thr Thr Phe Asn
        3120                3125                3130

cct gac cag ata ttg ctg gcc gat acc gac ggt tcc ggt acc acg gac    11484
Pro Asp Gln Ile Leu Leu Ala Asp Thr Asp Gly Ser Gly Thr Thr Asp
    3135                3140                3145

ctg att tat gcg atg agt gac cgg tta gtc att tat ttc aac cag agt    11532
Leu Ile Tyr Ala Met Ser Asp Arg Leu Val Ile Tyr Phe Asn Gln Ser
3150                3155                3160                3165

ggt aat tat ttc gcc gag ccg cat acg ctg ctc ttg ccg aaa ggt gtg    11580
Gly Asn Tyr Phe Ala Glu Pro His Thr Leu Leu Leu Pro Lys Gly Val
                3170                3175                3180

cgc tat gat cgc acc tgc agt ctg caa gtg gcg gat atc cag ggg ctg    11628
Arg Tyr Asp Arg Thr Cys Ser Leu Gln Val Ala Asp Ile Gln Gly Leu
            3185                3190                3195

ggg gtg cct agc ctg tta ctg acg gtc ccc cat gtc gcg cct cat cac    11676
Gly Val Pro Ser Leu Leu Leu Thr Val Pro His Val Ala Pro His His
        3200                3205                3210

tgg gtg tgc cat tta tcg gca gac aaa ccc tgg ttg ttg aat ggc atg    11724
Trp Val Cys His Leu Ser Ala Asp Lys Pro Trp Leu Leu Asn Gly Met
    3215                3220                3225

aac aac aat atg ggg gcc cgg cat gca ctg cac tat cgc agt tcg gtg    11772
Asn Asn Asn Met Gly Ala Arg His Ala Leu His Tyr Arg Ser Ser Val
3230                3235                3240                3245

cag ttc tgg ctg gat gag aaa gcc gag gca ctg gcg gca ggc agt tcc    11820
Gln Phe Trp Leu Asp Glu Lys Ala Glu Ala Leu Ala Ala Gly Ser Ser
                3250                3255                3260

cct gcc tgc tac ctg cca ttt aca ttg cat acc ctg tgg cgt tcg gtg    11868
Pro Ala Cys Tyr Leu Pro Phe Thr Leu His Thr Leu Trp Arg Ser Val
            3265                3270                3275

gtg cag gat gag atc acc ggt aac cgt ctg gtc agc gac gtg ctt tat    11916
Val Gln Asp Glu Ile Thr Gly Asn Arg Leu Val Ser Asp Val Leu Tyr
        3280                3285                3290

cgc cac ggc gtc tgg gac ggg cag gaa cgc gag ttt cgg ggg ttt ggt    11964
Arg His Gly Val Trp Asp Gly Gln Glu Arg Glu Phe Arg Gly Phe Gly
    3295                3300                3305

ttt gtt gag atc agg gat acc gat acc ttg gca agc cag ggt acg gcg    12012
Phe Val Glu Ile Arg Asp Thr Asp Thr Leu Ala Ser Gln Gly Thr Ala
3310                3315                3320                3325

acg gaa ctg agt atg cct tct gtg agc cgg aac tgg tat gcc acc ggg    12060
Thr Glu Leu Ser Met Pro Ser Val Ser Arg Asn Trp Tyr Ala Thr Gly
                3330                3335                3340

gta ccg gca gta gac gag cgt ctg ccg gag acg tat tgg caa aac gat    12108
Val Pro Ala Val Asp Glu Arg Leu Pro Glu Thr Tyr Trp Gln Asn Asp
            3345                3350                3355

gcc gcc gct ttt gcc gat ttc gcg acc cgt ttc act gtc ggt tca gga    12156
Ala Ala Ala Phe Ala Asp Phe Ala Thr Arg Phe Thr Val Gly Ser Gly
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        3360                3365                3370

gag gat gag cag aca tat act ccg gac gac agc aag aca ttc tgg ttg     12204
Glu Asp Glu Gln Thr Tyr Thr Pro Asp Asp Ser Lys Thr Phe Trp Leu
    3375                3380                3385

cag cga gcc ctg aaa ggc atc ctg ctg cgc agt gag tta tac ggt gcc     12252
Gln Arg Ala Leu Lys Gly Ile Leu Leu Arg Ser Glu Leu Tyr Gly Ala
3390                3395                3400                3405

gat ggc agc agc cag gcc gat atc cct tac agc gtc act gag tct cgc     12300
Asp Gly Ser Ser Gln Ala Asp Ile Pro Tyr Ser Val Thr Glu Ser Arg
                3410                3415                3420

ccg cag gta cgg cta gtt gaa gcg aat gga gac tac ccg gtg gtg tgg     12348
Pro Gln Val Arg Leu Val Glu Ala Asn Gly Asp Tyr Pro Val Val Trp
            3425                3430                3435

ccg atg ggc gcg gaa agc cgt acg tca gtt tat gaa cgg tac cac aat     12396
Pro Met Gly Ala Glu Ser Arg Thr Ser Val Tyr Glu Arg Tyr His Asn
        3440                3445                3450

gat cct caa tgc caa cag cag gcg gta ctc ctc agt gat gaa tac ggt     12444
Asp Pro Gln Cys Gln Gln Gln Ala Val Leu Leu Ser Asp Glu Tyr Gly
    3455                3460                3465

ttc cca ctg cgt cag gtc agt gtc aat tat cca cga cgc cct ccg tcg     12492
Phe Pro Leu Arg Gln Val Ser Val Asn Tyr Pro Arg Arg Pro Pro Ser
3470                3475                3480                3485

gcg gac aat cca tat ccg gcg tcc tta ccg gcg acg ctg ttc gcc aac     12540
Ala Asp Asn Pro Tyr Pro Ala Ser Leu Pro Ala Thr Leu Phe Ala Asn
                3490                3495                3500

agt tat gac gag cag cag cag ata tta cgc ctg ggg ttg caa cag agc     12588
Ser Tyr Asp Glu Gln Gln Gln Ile Leu Arg Leu Gly Leu Gln Gln Ser
            3505                3510                3515

agt gca cat cac ctt gtt tca ctg tct gag ggg cat tgg ttg ttg ggg     12636
Ser Ala His His Leu Val Ser Leu Ser Glu Gly His Trp Leu Leu Gly
        3520                3525                3530

ttg gcg gag gcg tcg cgg gac gat gta ttc acg tac tct gcg gac aac     12684
Leu Ala Glu Ala Ser Arg Asp Asp Val Phe Thr Tyr Ser Ala Asp Asn
    3535                3540                3545

gtg ccg gaa ggg ggt ctg acg ctg gaa cac ctg ttg gcg ccc gaa agc     12732
Val Pro Glu Gly Gly Leu Thr Leu Glu His Leu Leu Ala Pro Glu Ser
3550                3555                3560                3565

ctg gtc tcg gat agt cag gtc ggt acg ctg gcg ggt cag cag caa gtc     12780
Leu Val Ser Asp Ser Gln Val Gly Thr Leu Ala Gly Gln Gln Gln Val
                3570                3575                3580

tgg tat ctg gat tca caa gac gtt gcc acc gtc gct gct ccg cca ctc     12828
Trp Tyr Leu Asp Ser Gln Asp Val Ala Thr Val Ala Ala Pro Pro Leu
            3585                3590                3595

ccc ccc aag gta gct ttt atc gaa acg gcc gtg ctg gat gag ggt atg     12876
Pro Pro Lys Val Ala Phe Ile Glu Thr Ala Val Leu Asp Glu Gly Met
        3600                3605                3610

gtc agt tca ctg gct gcc tac att gtg gat gaa cat ctc gag caa gcc     12924
Val Ser Ser Leu Ala Ala Tyr Ile Val Asp Glu His Leu Glu Gln Ala
    3615                3620                3625

ggt tac cgg caa tcc gga tac ctt ttc cct cga ggc agg gaa gca gaa     12972
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Gly Tyr Arg Gln Ser Gly Tyr Leu Phe Pro Arg Gly Arg Glu Ala Glu
3630 3635 3640 3645

cag gca ttg tgg acc cag tgt cag gga tat gtt acc tat gcc ggc gca 13020
Gln Ala Leu Trp Thr Gln Cys Gln Gly Tyr Val Thr Tyr Ala Gly Ala
3650 3655 3660

gag cat ttc tgg cta ccg cta tcc ttt cgg gac agt atg ttg acc ggc 13068
Glu His Phe Trp Leu Pro Leu Ser Phe Arg Asp Ser Met Leu Thr Gly
3665 3670 3675

cca gtt acc gtg acg cgt gac gcg tac gac tgc gtc atc acg cag tgg 13116
Pro Val Thr Val Thr Arg Asp Ala Tyr Asp Cys Val Ile Thr Gln Trp
3680 3685 3690

cag gat gcc gca ggg att gtc acc aca gcc gac tat gac tgg cgc ttc 13164
Gln Asp Ala Ala Gly Ile Val Thr Thr Ala Asp Tyr Asp Trp Arg Phe
3695 3700 3705

ctg acg ccc gtc cgg gtg acg gac ccc aat gat aat ctg cag tcc gtc 13212
Leu Thr Pro Val Arg Val Thr Asp Pro Asn Asp Asn Leu Gln Ser Val
3710 3715 3720 3725

act ctg gat gct ctg ggc cgg gtg acc acc ctg cga ttc tgg ggc acg 13260
Thr Leu Asp Ala Leu Gly Arg Val Thr Thr Leu Arg Phe Trp Gly Thr
3730 3735 3740

gag aat ggt att gcc acc ggt tac agt gat gcc acg ttg tcc gtt ccg 13308
Glu Asn Gly Ile Ala Thr Gly Tyr Ser Asp Ala Thr Leu Ser Val Pro
3745 3750 3755

gac ggc gca gca gcc gct ctg gcg ttg acg gcg ccc cta cca gta gca 13356
Asp Gly Ala Ala Ala Ala Leu Ala Leu Thr Ala Pro Leu Pro Val Ala
3760 3765 3770

cag tgt ctg gtg tat gtc acg gac agt tgg gga gat gac gac aat gag 13404
Gln Cys Leu Val Tyr Val Thr Asp Ser Trp Gly Asp Asp Asp Asn Glu
3775 3780 3785

aaa atg ccc ccg cac gtg gtc gtg ctg gct acc gat cgc tat gac agt 13452
Lys Met Pro Pro His Val Val Val Leu Ala Thr Asp Arg Tyr Asp Ser
3790 3795 3800 3805

gat acc gga cag cag gtc cgc caa cag gtg aca ttc agt gac ggt ttt 13500
Asp Thr Gly Gln Gln Val Arg Gln Gln Val Thr Phe Ser Asp Gly Phe
3810 3815 3820

ggg cgt gag ttg caa tcg gca acc cgg cag gcc gag ggc aac gcc tgg 13548
Gly Arg Glu Leu Gln Ser Ala Thr Arg Gln Ala Glu Gly Asn Ala Trp
3825 3830 3835

caa cga gga cgc gac ggc aaa ctg gtg acg gcc agt gac gga ttg ccg 13596
Gln Arg Gly Arg Asp Gly Lys Leu Val Thr Ala Ser Asp Gly Leu Pro
3840 3845 3850

gtc act gta gca acg aat ttc cgc tgg gcg gtc acc ggg agg gcg gag 13644
Val Thr Val Ala Thr Asn Phe Arg Trp Ala Val Thr Gly Arg Ala Glu
3855 3860 3865

tat gac aat aaa ggt ctg cct gtt cgg gtt tat cag ccg tat ttt ctg 13692
Tyr Asp Asn Lys Gly Leu Pro Val Arg Val Tyr Gln Pro Tyr Phe Leu
3870 3875 3880 3885

gac agt tgg caa tat gtc agt gat gac agt gcc cgc cag gac ctg tat 13740
Asp Ser Trp Gln Tyr Val Ser Asp Asp Ser Ala Arg Gln Asp Leu Tyr
3890 3895 3900

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gcc gac acg cac ttt tac gat ccg acg gca cgg gaa tgg cag gtt att    13788
Ala Asp Thr His Phe Tyr Asp Pro Thr Ala Arg Glu Trp Gln Val Ile
            3905                3910                3915

acg gca aaa ggt gaa cgg cga cag gtg ctg tat acc ccg tgg ttt gtg    13836
Thr Ala Lys Gly Glu Arg Arg Gln Val Leu Tyr Thr Pro Trp Phe Val
        3920                3925                3930

gtc agt gaa gac gag aat gat acc gtt ggg cta aac gac gca tcc tga    13884
Val Ser Glu Asp Glu Asn Asp Thr Val Gly Leu Asn Asp Ala Ser  *
    3935                3940                3945

ctgggaagga ggggggacg gtg atg agt ccg tcg ccc ctg aca ggc gct gcc 13937
                         Met Ser Pro Ser Pro Leu Thr Gly Ala Ala
                             3950                3955

ctg atg gag aca aag atg aaa ata cac tat cag gtt gcg gcg gtt gtg    13985
Leu Met Glu Thr Lys Met Lys Ile His Tyr Gln Val Ala Ala Val Val
    3960                3965                3970

ctg aca ggt gtt atg gtt tgg ggg ctt tcc cat tgg cgt tac acc gtc    14033
Leu Thr Gly Val Met Val Trp Gly Leu Ser His Trp Arg Tyr Thr Val
3975                3980                3985                3990

ggt tac cac gcg gca gat act caa tgg caa caa cgc cag gcc gaa cag    14081
Gly Tyr His Ala Ala Asp Thr Gln Trp Gln Gln Arg Gln Ala Glu Gln
                3995                4000                4005

gaa agg gcc gat gcg ttg gcc ctc ctg gca gca gaa acc cgg gaa aga    14129
Glu Arg Ala Asp Ala Leu Ala Leu Leu Ala Ala Glu Thr Arg Glu Arg
            4010                4015                4020

aag tgg gag cag caa cga cag act gac atg aac aag gtg gct ata cat    14177
Lys Trp Glu Gln Gln Arg Gln Thr Asp Met Asn Lys Val Ala Ile His
        4025                4030                4035

gct gaa gaa gaa ctg gct gct gcg cgt gac gct gcc gct gat gct cag    14225
Ala Glu Glu Glu Leu Ala Ala Ala Arg Asp Ala Ala Ala Asp Ala Gln
    4040                4045                4050

cgc act ggt cag cgc ctg cag cac acc gtt acc acc ctc cag cgg caa    14273
Arg Thr Gly Gln Arg Leu Gln His Thr Val Thr Thr Leu Gln Arg Gln
4055                4060                4065                4070

ctt gcc agt cgt gaa acc cgc cgc ctt tcc gca gct acc gct atc ggt    14321
Leu Ala Ser Arg Glu Thr Arg Arg Leu Ser Ala Ala Thr Ala Ile Gly
                4075                4080                4085

aca gac gac ctc gga ggc caa ccc ggc gtt ttg ttt gcc gaa ctg ttc    14369
Thr Asp Asp Leu Gly Gly Gln Pro Gly Val Leu Phe Ala Glu Leu Phe
            4090                4095                4100

cgc cgc gct gac cag aga gcg gga gag ctg gca gcg tat gct gac agg    14417
Arg Arg Ala Asp Gln Arg Ala Gly Glu Leu Ala Ala Tyr Ala Asp Arg
        4105                4110                4115

acc aga gtg aaa tgg cag gcc tgc ggg cgc gcc tat cag gcg gct acg    14465
Thr Arg Val Lys Trp Gln Ala Cys Gly Arg Ala Tyr Gln Ala Ala Thr
    4120                4125                4130

cac gaa gca gaa aaa taa ggcgatttag ccgttaagga aaagtgacgg           14513
His Glu Ala Glu Lys  *
4135

tgttttcgcg attaatatta acaggagatc ac atg agc aca tcc ttg ttc agt    14566
                                    Met Ser Thr Ser Leu Phe Ser
                                    4140                4145
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agc acc ccg tcg gtc gcg gtg ctc gac aac cgc ggc ctg ttg gtg cgg 14614
Ser Thr Pro Ser Val Ala Val Leu Asp Asn Arg Gly Leu Leu Val Arg
4150 4155 4160

gag ctg cag tac tac cgc cat ccg gat aca ccg gag gag acg gac gag 14662
Glu Leu Gln Tyr Tyr Arg His Pro Asp Thr Pro Glu Glu Thr Asp Glu
4165 4170 4175

cgt atc acc tgc cat cag cac gat gag cgc ggc agc ttg tca caa agc 14710
Arg Ile Thr Cys His Gln His Asp Glu Arg Gly Ser Leu Ser Gln Ser
4180 4185 4190

gcc gac ccg cgg tta cac gcg gcc ggt ctg aca aat ttc acg tac ctg 14758
Ala Asp Pro Arg Leu His Ala Ala Gly Leu Thr Asn Phe Thr Tyr Leu
4195 4200 4205 4210

aat agc ctg acc ggg aca gta ctg cag agc gtc agc gcc gat gcc ggt 14806
Asn Ser Leu Thr Gly Thr Val Leu Gln Ser Val Ser Ala Asp Ala Gly
4215 4220 4225

acg tcg ctg gaa ctg agc gat gcc gcc ggg cgg gcg ttt ctg gcc gtc 14854
Thr Ser Leu Glu Leu Ser Asp Ala Ala Gly Arg Ala Phe Leu Ala Val
4230 4235 4240

acc ggg gct ggg acg gaa gac gcg gtc acc cgc acc tgg caa tat gaa 14902
Thr Gly Ala Gly Thr Glu Asp Ala Val Thr Arg Thr Trp Gln Tyr Glu
4245 4250 4255

gac gat acc ctg ccg ggc cgc ccg ctg agc atc acc gag cag gtt acc 14950
Asp Asp Thr Leu Pro Gly Arg Pro Leu Ser Ile Thr Glu Gln Val Thr
4260 4265 4270

ggt gaa gcc gcc caa att acg gaa cgc ttc gtg tac gct ggc aat acg 14998
Gly Glu Ala Ala Gln Ile Thr Glu Arg Phe Val Tyr Ala Gly Asn Thr
4275 4280 4285 4290

gat gcc gag aag att ctc aat ctg gct ggc cag tgt gtc agt cat tac 15046
Asp Ala Glu Lys Ile Leu Asn Leu Ala Gly Gln Cys Val Ser His Tyr
4295 4300 4305

gat acc gcc gga ctg gtg cag acg gac agc atc gcc ctg agc ggc gtg 15094
Asp Thr Ala Gly Leu Val Gln Thr Asp Ser Ile Ala Leu Ser Gly Val
4310 4315 4320

ccg ctc gcc gtc acg cgg cag ttg ctg ccc gac gcg gcg ggg gcc aac 15142
Pro Leu Ala Val Thr Arg Gln Leu Leu Pro Asp Ala Ala Gly Ala Asn
4325 4330 4335

tgg atg ggt gag gat gcc tcg gcc tgg aat gac ctg ctg gat ggg gag 15190
Trp Met Gly Glu Asp Ala Ser Ala Trp Asn Asp Leu Leu Asp Gly Glu
4340 4345 4350

acg ttc ttc acc cag acc cac gct gat gcg acc ggc gcc gtc ctg agc 15238
Thr Phe Phe Thr Gln Thr His Ala Asp Ala Thr Gly Ala Val Leu Ser
4355 4360 4365 4370

atc acc gat gca aaa ggt aat ctg cag cgt gtg gca tat gat gtg gct 15286
Ile Thr Asp Ala Lys Gly Asn Leu Gln Arg Val Ala Tyr Asp Val Ala
4375 4380 4385

ggg ctg cta tcg ggc agt tgg ttg acg ctg aag gac ggc acg gag cag 15334
Gly Leu Leu Ser Gly Ser Trp Leu Thr Leu Lys Asp Gly Thr Glu Gln
4390 4395 4400

gtc atc gtg gcc tcc ctg acg tac tcg gcc gcc ggg aaa aag ttg cgt 15382
Val Ile Val Ala Ser Leu Thr Tyr Ser Ala Ala Gly Lys Lys Leu Arg

4405 4410 4415

gaa gaa cac ggc aac ggc gtg gta acc tcg tat att tac gag ccg gaa 15430
Glu Glu His Gly Asn Gly Val Val Thr Ser Tyr Ile Tyr Glu Pro Glu
4420 4425 4430

aca cag cgc ctg acg ggg att aaa acg gaa cgt ccg tct ggg cac gtt 15478
Thr Gln Arg Leu Thr Gly Ile Lys Thr Glu Arg Pro Ser Gly His Val
4435 4440 4445 4450

gcc gga gca aaa gtg ctg cag gac ctg cgc tat acg tat gac ccg gta 15526
Ala Gly Ala Lys Val Leu Gln Asp Leu Arg Tyr Thr Tyr Asp Pro Val
4455 4460 4465

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Gly Asn Val Leu Ser Val Asn Asn Asp Ala Glu Glu Thr Arg Phe Trp
4470 4475 4480

cgt aac cag aaa gtg gta ccg gag aat acg tac atc tac gac agc ctg 15622
Arg Asn Gln Lys Val Val Pro Glu Asn Thr Tyr Ile Tyr Asp Ser Leu
4485 4490 4495

tac cag ctg gtc agc gcc aca ggg cgt gag atg gcc aat gcc ggc cag 15670
Tyr Gln Leu Val Ser Ala Thr Gly Arg Glu Met Ala Asn Ala Gly Gln
4500 4505 4510

cag ggc aac gac tta cca tcc gct aca gcc ccc ctt cct aca gac agc 15718
Gln Gly Asn Asp Leu Pro Ser Ala Thr Ala Pro Leu Pro Thr Asp Ser
4515 4520 4525 4530

tct gcc tac acc aat tac acg cgc acc tac cgt tat gac cgt ggt ggc 15766
Ser Ala Tyr Thr Asn Tyr Thr Arg Thr Tyr Arg Tyr Asp Arg Gly Gly
4535 4540 4545

aac ctg acg cag atg cgc cac agt gcc cct gcc acg aac aat aat tat 15814
Asn Leu Thr Gln Met Arg His Ser Ala Pro Ala Thr Asn Asn Asn Tyr
4550 4555 4560

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Thr Thr Asp Ile Thr Val Ser Asp Arg Ser Asn Arg Ala Val Leu Ser
4565 4570 4575

acg ttg gcg gaa gtg ccg tca gat gtt gat atg ctg ttc agt gca gga 15910
Thr Leu Ala Glu Val Pro Ser Asp Val Asp Met Leu Phe Ser Ala Gly
4580 4585 4590

ggt cac cag aag cac ctg cag ccg ggg caa gca ctg gtg tgg acg cca 15958
Gly His Gln Lys His Leu Gln Pro Gly Gln Ala Leu Val Trp Thr Pro
4595 4600 4605 4610

cgt gga gaa ctg caa aag gtg aca ccg gtg gtg cgt gat ggg ggg gcg 16006
Arg Gly Glu Leu Gln Lys Val Thr Pro Val Val Arg Asp Gly Gly Ala
4615 4620 4625

gac gac agc gaa agc tat cgg tat gat gcg ggc agt cag cgt att atc 16054
Asp Asp Ser Glu Ser Tyr Arg Tyr Asp Ala Gly Ser Gln Arg Ile Ile
4630 4635 4640

aaa acc ggc acg cgg caa act ggc aac aac gtt cag aca cag cgg gta 16102
Lys Thr Gly Thr Arg Gln Thr Gly Asn Asn Val Gln Thr Gln Arg Val
4645 4650 4655

gtg tac ctg ccg ggg ctg gag tta cgt atc atg gca aat ggc gtg acg 16150
Val Tyr Leu Pro Gly Leu Glu Leu Arg Ile Met Ala Asn Gly Val Thr
4660 4665 4670

gaa aaa gaa agc ctg cag gtt att acg gtg ggc gag gct ggg cgg gca 16198

Glu Lys Glu Ser Leu Gln Val Ile Thr Val Gly Glu Ala Gly Arg Ala
4675 4680 4685 4690

caa gtg cgc gta ttg cac tgg gag atc ggc aag ccg gat gac ctc gat 16246
Gln Val Arg Val Leu His Trp Glu Ile Gly Lys Pro Asp Asp Leu Asp
4695 4700 4705

gag gac tcg gtg cgt tac agt tac gat aac ctg gtg ggc agc agc cag 16294
Glu Asp Ser Val Arg Tyr Ser Tyr Asp Asn Leu Val Gly Ser Ser Gln
4710 4715 4720

ctg gag ctg gac aga gag ggt tac ctt atc agt gag gag gag ttc tac 16342
Leu Glu Leu Asp Arg Glu Gly Tyr Leu Ile Ser Glu Glu Glu Phe Tyr
4725 4730 4735

ccg tat ggc gga acg gct gtt ctg acg gcg cga agt gag gtt gag gct 16390
Pro Tyr Gly Gly Thr Ala Val Leu Thr Ala Arg Ser Glu Val Glu Ala
4740 4745 4750

gac tac aaa act atc cga tac tca ggc aag gag cgt gac gcg acg ggg 16438
Asp Tyr Lys Thr Ile Arg Tyr Ser Gly Lys Glu Arg Asp Ala Thr Gly
4755 4760 4765 4770

ctg gat tat tac ggt tat cgg tat tac cag cca tgg gca ggg cgc tgg 16486
Leu Asp Tyr Tyr Gly Tyr Arg Tyr Tyr Gln Pro Trp Ala Gly Arg Trp
4775 4780 4785

ctc tcc acg gac ccg gca ggc acg gtg gac ggg ctg aac ctg ttc cgc 16534
Leu Ser Thr Asp Pro Ala Gly Thr Val Asp Gly Leu Asn Leu Phe Arg
4790 4795 4800

atg gtg cgg aat aat ccc gtc acg ctg ttt gac agc aac ggg cgg atc 16582
Met Val Arg Asn Asn Pro Val Thr Leu Phe Asp Ser Asn Gly Arg Ile
4805 4810 4815

agt act ggt cag gag gcc aga cga tta gtg ggg gaa gca ttt gtt cat 16630
Ser Thr Gly Gln Glu Ala Arg Arg Leu Val Gly Glu Ala Phe Val His
4820 4825 4830

ccg tta cac atg cct gtt ttt gaa aga att tct gta gag aga aag att 16678
Pro Leu His Met Pro Val Phe Glu Arg Ile Ser Val Glu Arg Lys Ile
4835 4840 4845 4850

tca atg agc gta agg gaa gct ggc att tat act att tca gcg ctg ggt 16726
Ser Met Ser Val Arg Glu Ala Gly Ile Tyr Thr Ile Ser Ala Leu Gly
4855 4860 4865

gaa ggt gca gca gca aaa ggc cat aat att cta gag aaa acc att aaa 16774
Glu Gly Ala Ala Ala Lys Gly His Asn Ile Leu Glu Lys Thr Ile Lys
4870 4875 4880

ccc ggt tcc ctg aag gct atc tat ggt gat aaa gct gag tca att ctt 16822
Pro Gly Ser Leu Lys Ala Ile Tyr Gly Asp Lys Ala Glu Ser Ile Leu
4885 4890 4895

gga ctg gca aaa cgt agc ggt ctc gtt ggc cga gta gga cag tgg gat 16870
Gly Leu Ala Lys Arg Ser Gly Leu Val Gly Arg Val Gly Gln Trp Asp
4900 4905 4910

gca tca ggt gta cgt gga att tat gcg cac aac aga ccg ggt ggt gag 16918
Ala Ser Gly Val Arg Gly Ile Tyr Ala His Asn Arg Pro Gly Gly Glu
4915 4920 4925 4930

gat ttg gtt tat cct gtc agc ctg cag aat act tct gcc aat gaa att 16966
Asp Leu Val Tyr Pro Val Ser Leu Gln Asn Thr Ser Ala Asn Glu Ile
4935 4940 4945

gtt aat gca tgg ata aaa ttt aaa atc atc acg ccc tac acc ggg gat 17014
Val Asn Ala Trp Ile Lys Phe Lys Ile Ile Thr Pro Tyr Thr Gly Asp
4950 4955 4960

tat gac atg cac gat att att aaa ttc tct gat ggg aaa ggg cat gtg 17062
Tyr Asp Met His Asp Ile Ile Lys Phe Ser Asp Gly Lys Gly His Val
4965 4970 4975

cct aca gcg gaa agt agt gag gaa aga gga gta aaa gat cta att aat 17110
Pro Thr Ala Glu Ser Ser Glu Glu Arg Gly Val Lys Asp Leu Ile Asn
4980 4985 4990

aaa ggt gtt gcg gag gtc gat cct tcc aga ccc ttt gag tat aca gcg 17158
Lys Gly Val Ala Glu Val Asp Pro Ser Arg Pro Phe Glu Tyr Thr Ala
4995 5000 5005 5010

atg aat gtt att cgc cat gga cca cag gtg aac ttt gtt ccc tat atg 17206
Met Asn Val Ile Arg His Gly Pro Gln Val Asn Phe Val Pro Tyr Met
5015 5020 5025

tgg gaa cat gag cac gat aaa gtc gtt aat gat aat ggt tat ctg ggg 17254
Trp Glu His Glu His Asp Lys Val Val Asn Asp Asn Gly Tyr Leu Gly
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gtg gta gct agc ccg ggg ccg ttc ccg gta gcg atg gta cat cag ggg 17302
Val Val Ala Ser Pro Gly Pro Phe Pro Val Ala Met Val His Gln Gly
5045 5050 5055

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Glu Trp Thr Val Phe Asp Asn Ser Glu Glu Leu Phe Asn Phe Tyr Lys
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Ser Thr Asn Thr Pro Leu Pro Glu His Trp Ser Gln Asp Phe Met Asp
5075 5080 5085 5090

aga ggg aaa gga ata gtc gca act cct cgg cat gct gaa ctt ctt gat 17446
Arg Gly Lys Gly Ile Val Ala Thr Pro Arg His Ala Glu Leu Leu Asp
5095 5100 5105

aaa cga cga gtc atg tac taa tcgtaacgat ttcctgcctt acccaaagta 17497
Lys Arg Arg Val Met Tyr *
5110

tacagcccgg tgagacattt tctctgtctc atttgggttg tttttgtctc atctgcatgt 17557
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<210> 2
<211> 144
<212> PRT
<213> Serratia entomophila

<220>
<223> ORF1 amino acid sequence encoding an insecticidal protein when
linked with at least SEQ ID NO: 1

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1 5 10 15
Leu Arg Leu His Ala Tyr Arg Cys Ala Ala Asp Val Trp Thr Val Gly
20 25 30
Tyr Gly His Thr Ala Gly Val Thr Lys Gly Asp Ile Ile Thr Val Asp
35 40 45
Glu Ala Gln Thr Met Leu Thr Asn Asp Ile Thr Val Phe Glu Arg Ala
50 55 60
Val Ser Gln Ala Val Ala Val Pro Leu Asn Gln Ser Gln Tyr Asp Ala
65 70 75 80
Leu Val Ser Leu Val Phe Asn Ile Gly Gln Gly Asn Phe Lys Arg Ser
85 90 95
Thr Leu Leu Lys Lys Leu Asn Lys Gln Asp Tyr Val Gly Ala Gly Asn
100 105 110
Glu Phe Leu Arg Trp Thr Arg Ala Asn Gly Lys Val Leu Pro Gly Leu
115 120 125
Ile Arg Arg Arg Glu Ala Glu Arg Val Leu Phe Glu Lys Leu Gly Ala
130 135 140

<210> 3
<211> 191
<212> PRT
<213> Serratia entomophila

<220>
<223> ORF2 amino acid sequence encoding an insecticidal protein when
linked with at least SEQ ID NO: 1

<400> 3
Met Ser Pro Ser Pro Leu Thr Gly Ala Ala Leu Met Glu Thr Lys Met
1 5 10 15
Lys Ile His Tyr Gln Val Ala Ala Val Val Leu Thr Gly Val Met Val
20 25 30
Trp Gly Leu Ser His Trp Arg Tyr Thr Val Gly Tyr His Ala Ala Asp
35 40 45
Thr Gln Trp Gln Gln Arg Gln Ala Glu Gln Glu Arg Ala Asp Ala Leu
50 55 60
Ala Leu Leu Ala Ala Glu Thr Arg Glu Arg Lys Trp Glu Gln Gln Arg
65 70 75 80
Gln Thr Asp Met Asn Lys Val Ala Ile His Ala Glu Glu Glu Leu Ala
85 90 95
Ala Ala Arg Asp Ala Ala Ala Asp Ala Gln Arg Thr Gly Gln Arg Leu
100 105 110
Gln His Thr Val Thr Thr Leu Gln Arg Gln Leu Ala Ser Arg Glu Thr
115 120 125
Arg Arg Leu Ser Ala Ala Thr Ala Ile Gly Thr Asp Asp Leu Gly Gly
130 135 140
Gln Pro Gly Val Leu Phe Ala Glu Leu Phe Arg Arg Ala Asp Gln Arg
145 150 155 160
Ala Gly Glu Leu Ala Ala Tyr Ala Asp Arg Thr Arg Val Lys Trp Gln
165 170 175
Ala Cys Gly Arg Ala Tyr Gln Ala Ala Thr His Glu Ala Glu Lys
180 185 190

<210> 4
<211> 2376
<212> PRT
<213> Serratia entomophila

<220>
<223> SepA amino acid sequence encoding an insecticidal protein when linked with at least SEQ ID NO: 1

<400> 4
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1 5 10 15
Asn Ala Pro Arg Ala Arg Leu Ser Glu Glu Asn Asp Thr Ala Val Thr
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Leu Thr Asp Leu Phe Ser Arg Ser Phe Pro Glu Val Lys Lys Ile Thr
35 40 45
Gly Asp Ser Leu Ser Trp Gly Glu Val Cys Tyr Leu Tyr Ser Gln Ala
50 55 60
Gln His Glu Gln Lys Glu Asn Arg Leu Thr Glu Ser Arg Ile Leu Ala
65 70 75 80
Arg Ala Asn Pro Leu Leu Val Asn Ala Val Arg Leu Gly Ile Arg Gln
85 90 95
Ala Ala Gly Ser Arg Ser Tyr Asp Asp Trp Phe Gly Ser Arg Ala Asp
100 105 110
Arg Phe Ala Arg Pro Gly Ser Val Ala Ser Met Phe Ser Pro Ala Ala
115 120 125
Tyr Leu Thr Glu Leu Tyr Arg Glu Ala Lys Asp Leu His Pro Asp Thr
130 135 140
Ser Leu Phe Arg Leu Asp Ile Arg Arg Pro Asp Leu Ala Ala Leu Ala
145 150 155 160
Leu Ser Gln Asn Asn Met Asp Asp Glu Leu Ser Thr Leu Ser Leu Ser
165 170 175
Asn Glu Leu Leu Tyr Arg Gly Ile Gly Ala Ala Glu Gly Leu Asp Asp
180 185 190
Asp Ser Val Arg Glu Leu Leu Ala Gly Tyr Arg Leu Thr Gly Leu Thr
195 200 205
Pro Tyr His Trp Ala Tyr Glu Ala Ala Arg Gln Ala Ile Leu Val Gln
210 215 220
Asp Pro Thr Leu Met Gly Phe Ser Arg Asn Pro Asp Val Ala Gln Leu
225 230 235 240
Met Asp Pro Ala Ser Met Leu Ala Ile Glu Ala Asp Ile Ser Pro Glu
245 250 255
Leu Tyr Gln Ile Leu Ala Glu Glu Ile Thr Thr Asp Ser Tyr Glu Ala
260 265 270
Leu Trp Ser Lys Asn Phe Gly Asp Met Pro Pro Ser Ser Leu Leu Ser
275 280 285
Tyr Asp Ala Leu Ala Thr Phe Tyr Asp Leu Asp Tyr Asp Glu Leu Thr
290 295 300
Ser Leu Leu Ser Leu Arg Leu Asp Phe Ser Asn Pro Asn Asn Glu Tyr
305 310 315 320
Tyr Ile Asn Ser Gln Leu Ser Val Val Thr Leu Asn Glu Ser Thr Gly
325 330 335
Leu Ile Thr Ile His His Tyr Leu Arg Thr Leu Gly Gly Asp Ser Gln
340 345 350
Gln Ile Asn Pro Glu Leu Ile Pro Tyr Gly Asp Gly Thr Tyr Leu Tyr
355 360 365
Asn Phe Ser Val Val Ser Thr Ile Ser Glu Asp Ser Phe Lys Leu Gly
370 375 380
Ser Leu Gly Ser Asn Ser Ser Asn Leu Tyr Ser Gly Asp Tyr Gln Leu
385 390 395 400
Gln Lys Gly Val Arg Tyr Ser Ile Pro Val Glu Ile Asp Glu Gly Lys
405 410 415
Leu Asn Asp Gly Ile Thr Ile Gly Leu Ser Arg Lys Gly Gly Gly Tyr
420 425 430
Tyr Ser Thr Val Asn Phe Thr Leu Ile Glu Tyr Asp Pro Ala Ile Phe
435 440 445

Ile Leu Lys Leu Asn Lys Val Ile Arg Leu Tyr Lys Ala Thr Gly Met
450 455 460
Thr Thr Ala Glu Ile Tyr Gln Ile Thr Asn Ile Leu Asn Asn Gly Leu
465 470 475 480
Thr Ile Asp His Ala Val Leu Ser Lys Ile Phe Leu Val Arg Tyr Leu
485 490 495
Met Arg His Tyr Gln Leu Asp Val Ala Arg Ser Leu Ile Leu Cys Asn
500 505 510
Gly Thr Ile Ser Asp Gln Ala Phe Ser Gly Glu Thr Gly Leu Phe Thr
515 520 525
Thr Leu Phe Asn Thr Pro Pro Leu Asn Gly Gln Leu Phe Ser Ala Asp
530 535 540
Asp Thr Pro Leu Asp Leu Arg Ser Glu Ala Pro Glu Asp Ala Phe Arg
545 550 555 560
Leu Ser Val Leu Lys Arg Ala Phe Asn Ile Ser Ala Ser Gly Leu Ser
565 570 575
Thr Leu Trp Gln Leu Ala Ser Gly Asp Ser Ser Ala Gly Phe Ser Cys
580 585 590
Ser Ala Asp Asn Ile Ala Ala Leu Tyr Arg Val Lys Leu Leu Ala Asp
595 600 605
Ile His Asp Leu Ser Ala Gly Glu Leu Ser Met Leu Leu Ser Val Ser
610 615 620
Pro Phe Ser Gly Val Ala Ala Gly Ser Leu Ser Asp Asn Glu Leu Thr
625 630 635 640
Gln Phe Leu Tyr Gln Thr Thr Thr Trp Leu Thr Glu Gln Gly Trp Thr
645 650 655
Val Ser Asp Val Phe Leu Met Leu Thr Thr Gln Tyr Gly Thr Leu Leu
660 665 670
Thr Pro Asp Ile Glu Asn Leu Leu Ala Ser Leu Arg Asn Gly Leu Ser
675 680 685
Gly Arg Glu Leu Phe Pro Glu Thr Leu Pro Gly Asp Gly Ala Pro Phe
690 695 700
Ile Ala Ala Ala Met Gln Leu Asp Ala Thr Asp Thr Ala Lys Ala Met
705 710 715 720
Leu Thr Trp Ala Asp Gln Leu Lys Pro Glu Gly Leu Thr Leu Thr Glu
725 730 735
Phe Ile Leu Leu Val Met Asn Ala Ala Pro Asn Asp Glu Gln Ala Gly
740 745 750
Gln Met Ala Gly Phe Cys Gln Ala Leu Trp Gln Leu Ala Leu Ile Ile
755 760 765
Arg Ser Thr Gly Leu Ser Thr Arg Glu Leu Thr Leu Leu Val Ser Gln
770 775 780
Pro Gly Arg Phe Arg Thr Gly Trp His His Leu Pro His Asp Leu Pro
785 790 795 800
Ala Leu Arg Asp Ile Thr Arg Phe His Ala Val Val Asn Arg Ser Gly
805 810 815
Ser His Ala Gly Glu Val Leu Thr Ala Leu Glu Thr Gly Glu Leu Ser
820 825 830
Ser Ala Leu Leu Ala Arg Ala Leu Ser Gln Asn Glu Gln Asp Val Thr
835 840 845
Gly Ala Leu Ala Gln Val Arg Gly Ala Gly Glu Gln Asp Asn Ser Val
850 855 860
Phe Thr Ser Trp Glu Glu Val Asp Gln Ala Glu Gln Trp Leu Asp Met
865 870 875 880
Ser Glu Thr Leu Ser Ile Thr Pro Ser Gly Leu Ala Ser Leu Ile Ala
885 890 895
Leu Lys Tyr Ile Asn Val Ser Asp Asp Ser Ala Pro Leu Tyr Ser Gln
900 905 910
Trp Gln Val Val Ser Gly Leu Leu Gln Ala Gly Leu Lys Ser Ser Gln
915 920 925
Ser Ser Ala Leu His Asp Tyr Leu Glu Glu Gly Thr Ser Ser Ala Leu
930 935 940
Cys Ala Tyr Tyr Leu Arg Asn Leu Ala Pro Asn Met Val Ser Gly Arg
945 950 955 960
Asp Asp Leu Phe Gly Tyr Leu Leu Leu Asp Asn Gln Val Ser Ala Lys
965 970 975
Val Lys Thr Thr Arg Ile Ala Glu Ala Ile Ala Gly Ile Arg Leu Tyr

980 985 990
Ile Asn Arg Ala Leu Asn Gly Ile Glu Leu Ser Ala Met Ala Glu Val
995 1000 1005
Arg Gly Arg Gln Phe Phe Thr Asp Trp Asp Thr Phe Asn Lys Arg Tyr
1010 1015 1020
Ser Thr Trp Ala Gly Val Ser Glu Leu Val Tyr Tyr Pro Glu Asn Tyr
1025 1030 1035 1040
Leu Asp Pro Thr Val Arg Ile Gly Gln Thr Gly Met Met Asp Thr Leu
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Leu Gln Ser Val Ser Gln Ser Ser Ile Asn Arg Asp Thr Val Glu Asp
1060 1065 1070
Ala Phe Lys Thr Tyr Leu Thr Thr Phe Glu Gln Ile Ala Asn Leu Asn
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1105 1110 1115 1120
Ser Ala Asn His Ser Lys Ile Gln Asp Ser Met Met Pro Ala Asn Ala
1125 1130 1135
Trp Thr Gly Trp Thr Lys Ile Asn Cys Gly Met Asn Pro Trp Ser Asp
1140 1145 1150
Leu Val Cys Ser Val Phe Phe Asn Ser Arg Leu Tyr Val Val Trp Val
1155 1160 1165
Glu Glu Asn Gln Ser Ala Asp Thr Glu Ala Glu Ser Thr Thr Thr Thr
1170 1175 1180
Gln Gln Ser Tyr Thr Leu Lys Leu Ser Phe Arg Arg Tyr Asp Gly Thr
1185 1190 1195 1200
Trp Ser Ser Pro Val Ser Phe Asp Ile Thr Gly Asn Ile Ala Phe Pro
1205 1210 1215
Glu Thr Gln Gly Met His Val Thr Cys Asn Pro Leu Thr Glu Gln Leu
1220 1225 1230
Tyr Cys Ala Phe Tyr Ser Val Thr Ser Lys Pro Asp Phe Asp Asn Ala
1235 1240 1245
Gln Leu Ile Ser Val Asp Asn Asp Met Thr Leu Asn Val Ile Ser Asp
1250 1255 1260
Ile Gly Ile Phe Lys Ser Val Ser His Glu Phe Asn Thr Ser Thr Glu
1265 1270 1275 1280
Lys Phe Ile Asn Asn Val Phe Ser Asp Pro Ser Ala Asn Tyr Phe Val
1285 1290 1295
Ser Ala Thr Ser Leu Ile Asp Asp Val Ile His Ser Asp Phe Ser Leu
1300 1305 1310
Leu Asn Ser Lys Thr Thr Ser Thr Val Phe Thr Asn Glu Asp Ser Ser
1315 1320 1325
Leu Leu Thr Pro Glu Leu His Ile Thr Ala Asn Val Ser Cys Phe Val
1330 1335 1340
Ser Thr Ala Gly Ile Ala Thr Gln Ser Thr Ile Glu Lys Phe Val Gln
1345 1350 1355 1360
Ala Gly Ile Glu Phe Glu Glu Ile Asn Phe Tyr Ala Gly Gln Ala Ala
1365 1370 1375
Gly Gly Phe Asp Gly Phe Val Gly Val Asp Val Ser Asn Ser Lys Val
1380 1385 1390
Tyr Gln Val Gly Lys Glu Ala Val Gly Val Thr Val Lys Ser Tyr Ser
1395 1400 1405
Val Thr Gly Val Ser Gly Ser Val Glu Leu Phe Ile Asp Ser Ser Asn
1410 1415 1420
Lys Tyr Phe Ser Gly Ile Leu Ser Asp Lys Met Ile Thr Ala Leu Ile
1425 1430 1435 1440
Ser Gly Ser Thr Ser Lys Val Asn Tyr Val Ser Ser Ile Gly Ser Gln
1445 1450 1455
Asp Phe Trp Ser Val Lys Ser Leu Met Pro Ala Leu Gln Ile Tyr Glu
1460 1465 1470
Leu Ile Asp Asp Ile Ile Leu Thr Ser Gly Val Asn Gly Thr Glu Ile
1475 1480 1485
Lys Ser Trp Pro Ser Ala Glu Trp Tyr Asn Asp Lys Leu Ser Leu Gln
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Ser Gly Asn Asn Leu Phe Asn Thr Lys Ser Leu Ser Phe Thr Val Asn
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Thr Ser Asp Ile Val Glu Asp Glu Phe Asp Val Thr Phe Thr Phe Thr
1525 1530 1535
Ala Val Asp Gln Asn Asn Val Val Leu Ala Ala Arg Thr Ala Ile Leu
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Thr Val Ile Arg Asn Ile Asn Asn Asp Thr Ser Val Ile Ala Leu Arg
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Lys Asn Thr Arg Gly Ala Gln Tyr Ile Arg Phe Thr Ala Gly Asn Asp
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1620 1625 1630
Ser Gly Ala Asn Ala Leu Tyr Phe Trp Glu Leu Phe Tyr Tyr Thr Pro
1635 1640 1645
Met Met Val Phe Gln Arg Leu Leu Gln Glu Gln His Phe Pro Glu Ala
1650 1655 1660
Thr Arg Trp Leu Gln Tyr Val Trp Asn Pro Ala Gly His Val Val Asn
1665 1670 1675 1680
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1685 1690 1695
Thr Gly Trp Asn Asp Ser Pro Leu Asp Ser Ile Asp Pro Asp Ala Ile
1700 1705 1710
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Arg Asp Thr Leu Asn Glu Ala Arg Met Trp Tyr Val Gln Ala Leu Asn
1745 1750 1755 1760
Leu Leu Gly Asp Glu Pro Tyr Ile Ser Phe Asp Ala Asp Trp Ser Ala
1765 1770 1775
Leu Thr Leu Gly Asp Ala Ala Ser Glu Val Thr Arg Arg Asp Tyr Gln
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Glu Ala Leu Leu Ala Val Arg Arg Leu Val Pro Ala Pro Glu Thr Arg
1795 1800 1805
Thr Ala Asn Ser Leu Thr Ala Leu Phe Leu Pro Gln Gln Asn Glu Val
1810 1815 1820
Leu Lys Gly Tyr Trp Gln Thr Leu Ala Gln Arg Leu His Asn Leu Arg
1825 1830 1835 1840
His Asn Leu Ser Ile Asp Gly Gln Pro Leu Ser Leu Ser Val Tyr Ala
1845 1850 1855
Thr Pro Ser Glu Pro Ser Ala Leu Gln Ser Ala Val Val Asn Ser Ala
1860 1865 1870
Gln Gly Ala Ala Ala Leu Pro Ala Ala Val Met Pro Leu Tyr Ser Phe
1875 1880 1885
Pro Val Met Leu Glu Asn Ala Arg Gly Met Val Ser Leu Leu Thr Gly
1890 1895 1900
Phe Gly Asn Thr Leu Leu Gly Ile Thr Glu Arg Gln Asp Ala Glu Ala
1905 1910 1915 1920
Leu Ala Lys Leu Leu Gln Thr Gln Gly Ser Glu Leu Ile Arg Gln Gly
1925 1930 1935
Leu Arg Gln Gln Asp Asn Val Leu Glu Glu Ile Asp Ala Asp Ile Ala
1940 1945 1950
Ala Leu Glu Glu Ser Arg Arg Gly Ala Gln Met Arg Phe Glu Arg Tyr
1955 1960 1965
Lys Val Leu Tyr Glu Ala Asp Val Asn Thr Gly Glu Lys Gln Ala Met
1970 1975 1980
Asp Leu Tyr Leu Ser Ser Ser Val Leu Ser Ala Ser Thr Ala Ala Leu
1985 1990 1995 2000
Phe Leu Ala Glu Ala Ala Ala Asp Met Leu Pro Asn Ile Tyr Gly Leu
2005 2010 2015
Ala Val Gly Gly Ser Arg Tyr Gly Ala Leu Phe Lys Ala Thr Ala Ile
2020 2025 2030
Gly Ile Gln Val Ser Ser Asp Ala Thr Arg Ile Ser Ala Asp Lys Ile
2035 2040 2045
Ser Gln Ser Glu Val Tyr Arg Arg Arg Arg Glu Glu Trp Glu Ile Gln

2050 2055 2060
Arg Asp Ser Ala Gln Ser Asp Val Ala Gln Ile Asp Ala Gln Leu Ala
2065 2070 2075 2080
Ala Met Ala Val Arg Arg Glu Gly Ala Glu Leu Gln Lys Thr Tyr Leu
2085 2090 2095
Glu Thr Gln Gln Thr Gln Ala Gln Ala Gln Leu Ala Phe Leu Gln Ser
2100 2105 2110
Lys Phe Asn Asn Thr Ala Leu Tyr Ser Trp Leu Arg Gly Arg Leu Ser
2115 2120 2125
Ala Ile Tyr Tyr Gln Phe Tyr Asp Leu Ala Val Ser Arg Cys Leu Met
2130 2135 2140
Ala Gln Gln Ala Trp Gln Trp Asp Lys Phe Glu Thr Arg Ser Phe Ile
2145 2150 2155 2160
Gln Pro Gly Ala Trp Met Gly Ala Asn Ala Gly Leu Leu Ala Gly Glu
2165 2170 2175
Thr Leu Met Leu Asn Leu Ala Gln Met Glu Gln Ala Trp Leu Thr Gly
2180 2185 2190
Asp Glu Arg Ala Ile Glu Val Thr Arg Thr Val Cys Leu Ser Glu Val
2195 2200 2205
Tyr Thr Ser Leu Ala Glu Asp Ala Ala Phe Ser Leu Ala Asp Lys Val
2210 2215 2220
Val Glu Leu Val Ser Asn Gly Ser Gly Ser Ala Gly Thr Lys Ser Asn
2225 2230 2235 2240
Gly Leu Gln Met Asp Gln Gln Gln Leu Glu Ala Thr Leu Lys Leu Ala
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Asp Leu Gly Ile Gly Asn Asp Tyr Pro Val Ser Leu Gly Thr Met Arg
2260 2265 2270
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Gln Asp Val Arg Ala Val Leu Ser Tyr Gly Gly Ser Met Val Met Pro
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2305 2310 2315 2320
Gln Phe Gln Leu Asp Phe Asn Asp Pro Arg Tyr Leu Pro Phe Glu Gly
2325 2330 2335
Leu Pro Val Asp Asp Thr Gly Thr Leu Thr Leu Ser Phe Pro Asp Ala
2340 2345 2350
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His Ile Arg Tyr Thr Ile Ile Ser
2370 2375

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<212> PRT
<213> Serratia entomophila

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linked with at least SEQ ID NO: 1

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35 40 45
Arg Gly Tyr Ala Pro Thr Gly Ala Leu Asn Tyr His Ser Arg Ser Gly
50 55 60
Asn Gly Pro Phe Gly Ile Gly Trp Gly Ile Gly Gly Ala Ala Val Gln
65 70 75 80
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85 90 95
Thr Gly Pro Asp Gly Glu Val Leu Val Pro Ala Leu Thr Ala Ala Gly
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Ser Arg Leu Glu Arg Trp Leu Pro Ala Asp Glu Thr Glu Thr Glu Phe
145                 150                 155                 160
Trp Val Leu Tyr Thr Pro Asp Gly Gln Val Ala Leu Leu Gly Arg Asn
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Ala Gln Ala Arg Ile Ser Asn Pro Thr Ala Pro Thr Gln Thr Ala Val
            180                 185                 190
Trp Leu Met Glu Ser Ser Val Ser Leu Thr Gly Glu Gln Met Tyr Tyr
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Ala His Pro Gln Ala Gly Ala Gln Arg Tyr Pro Val Ala Val Trp Tyr
225                 230                 235                 240
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Ser Met Asp Ser Trp Leu Phe Ile Leu Val Phe Asp Tyr Gly Glu Arg
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Glu Trp Leu Cys Arg Gln Asp Cys Phe Ser Gly Tyr Glu Phe Gly Phe
    290                 295                 300
Asn Leu Arg Thr Arg Arg Leu Cys Arg Gln Val Leu Met Phe His Tyr
305                 310                 315                 320
Leu Gly Val Leu Ala Gly Ser Ser Gly Ala Asn Asp Ala Pro Ala Leu
                325                 330                 335
Ile Ser Arg Leu Leu Leu Asp Tyr Arg Glu Ser Pro Ser Leu Ser Leu
            340                 345                 350
Leu Glu Asn Val His Gln Val Ala Tyr Glu Ser Asp Gly Thr Ser Cys
        355                 360                 365
Ala Leu Pro Ala Leu Ala Leu Gly Trp Gln Thr Phe Thr Pro Pro Thr
    370                 375                 380
Leu Ser Ala Trp Gln Thr Arg Asp Asp Met Gly Lys Leu Ser Leu Leu
385                 390                 395                 400
Gln Pro Tyr Gln Leu Val Asp Leu Asn Gly Glu Gly Val Val Gly Ile
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            420                 425                 430
Ser Gly Asp Asp Pro Asp Ala Val Thr Trp Gly Ala Ala Ala Ala Leu
        435                 440                 445
Pro Thr Met Pro Ala Leu His Asn Ser Gly Ile Leu Ala Asp Leu Asn
    450                 455                 460
Gly Asp Gly Arg Leu Glu Trp Val Val Thr Ala Pro Gly Val Ala Gly
465                 470                 475                 480
Met Tyr Asp Arg Thr Pro Gly Arg Asp Trp Leu His Phe Thr Pro Leu
                485                 490                 495
Ser Ala Leu Pro Val Glu Tyr Ala His Pro Lys Ala Val Leu Ala Asp
            500                 505                 510
Ile Leu Gly Ala Gly Leu Thr Asp Met Val Leu Ile Gly Pro Arg Ser
        515                 520                 525
Val Arg Leu Tyr Ser Gly Lys Asn Asp Gly Trp Asn Lys Gly Glu Thr
    530                 535                 540
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545                 550                 555                 560
Arg Thr Leu Val Ala Phe Ser Asp Met Ala Gly Ser Gly Gln Gln His
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His Gly Arg Phe Gly Gln Pro Val Asn Ile Pro Gly Phe Ser Gln Ser
        595                 600                 605
Val Thr Thr Phe Asn Pro Asp Gln Ile Leu Leu Ala Asp Thr Asp Gly
    610                 615                 620
Ser Gly Thr Thr Asp Leu Ile Tyr Ala Met Ser Asp Arg Leu Val Ile
625                 630                 635                 640
Tyr Phe Asn Gln Ser Gly Asn Tyr Phe Ala Glu Pro His Thr Leu Leu
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645 650 655
Leu Pro Lys Gly Val Arg Tyr Asp Arg Thr Cys Ser Leu Gln Val Ala
660 665 670
Asp Ile Gln Gly Leu Gly Val Pro Ser Leu Leu Leu Thr Val Pro His
675 680 685
Val Ala Pro His His Trp Val Cys His Leu Ser Ala Asp Lys Pro Trp
690 695 700
Leu Leu Asn Gly Met Asn Asn Asn Met Gly Ala Arg His Ala Leu His
705 710 715 720
Tyr Arg Ser Ser Val Gln Phe Trp Leu Asp Glu Lys Ala Glu Ala Leu
725 730 735
Ala Ala Gly Ser Ser Pro Ala Cys Tyr Leu Pro Phe Thr Leu His Thr
740 745 750
Leu Trp Arg Ser Val Val Gln Asp Glu Ile Thr Gly Asn Arg Leu Val
755 760 765
Ser Asp Val Leu Tyr Arg His Gly Val Trp Asp Gly Gln Glu Arg Glu
770 775 780
Phe Arg Gly Phe Gly Phe Val Glu Ile Arg Asp Thr Asp Thr Leu Ala
785 790 795 800
Ser Gln Gly Thr Ala Thr Glu Leu Ser Met Pro Ser Val Ser Arg Asn
805 810 815
Trp Tyr Ala Thr Gly Val Pro Ala Val Asp Glu Arg Leu Pro Glu Thr
820 825 830
Tyr Trp Gln Asn Asp Ala Ala Ala Phe Ala Asp Phe Ala Thr Arg Phe
835 840 845
Thr Val Gly Ser Gly Glu Asp Glu Gln Thr Tyr Thr Pro Asp Asp Ser
850 855 860
Lys Thr Phe Trp Leu Gln Arg Ala Leu Lys Gly Ile Leu Leu Arg Ser
865 870 875 880
Glu Leu Tyr Gly Ala Asp Gly Ser Ser Gln Ala Asp Ile Pro Tyr Ser
885 890 895
Val Thr Glu Ser Arg Pro Gln Val Arg Leu Val Glu Ala Asn Gly Asp
900 905 910
Tyr Pro Val Val Trp Pro Met Gly Ala Glu Ser Arg Thr Ser Val Tyr
915 920 925
Glu Arg Tyr His Asn Asp Pro Gln Cys Gln Gln Gln Ala Val Leu Leu
930 935 940
Ser Asp Glu Tyr Gly Phe Pro Leu Arg Gln Val Ser Val Asn Tyr Pro
945 950 955 960
Arg Arg Pro Pro Ser Ala Asp Asn Pro Tyr Pro Ala Ser Leu Pro Ala
965 970 975
Thr Leu Phe Ala Asn Ser Tyr Asp Glu Gln Gln Gln Ile Leu Arg Leu
980 985 990
Gly Leu Gln Gln Ser Ser Ala His His Leu Val Ser Leu Ser Glu Gly
995 1000 1005
His Trp Leu Leu Gly Leu Ala Glu Ala Ser Arg Asp Asp Val Phe Thr
1010 1015 1020
Tyr Ser Ala Asp Asn Val Pro Glu Gly Gly Leu Thr Leu Glu His Leu
1025 1030 1035 1040
Leu Ala Pro Glu Ser Leu Val Ser Asp Ser Gln Val Gly Thr Leu Ala
1045 1050 1055
Gly Gln Gln Gln Val Trp Tyr Leu Asp Ser Gln Asp Val Ala Thr Val
1060 1065 1070
Ala Ala Pro Pro Leu Pro Pro Lys Val Ala Phe Ile Glu Thr Ala Val
1075 1080 1085
Leu Asp Glu Gly Met Val Ser Ser Leu Ala Ala Tyr Ile Val Asp Glu
1090 1095 1100
His Leu Glu Gln Ala Gly Tyr Arg Gln Ser Gly Tyr Leu Phe Pro Arg
1105 1110 1115 1120
Gly Arg Glu Ala Glu Gln Ala Leu Trp Thr Gln Cys Gln Gly Tyr Val
1125 1130 1135
Thr Tyr Ala Gly Ala Glu His Phe Trp Leu Pro Leu Ser Phe Arg Asp
1140 1145 1150
Ser Met Leu Thr Gly Pro Val Thr Val Thr Arg Asp Ala Tyr Asp Cys
1155 1160 1165
Val Ile Thr Gln Trp Gln Asp Ala Ala Gly Ile Val Thr Thr Ala Asp
1170 1175 1180

Tyr Asp Trp Arg Phe Leu Thr Pro Val Arg Val Thr Asp Pro Asn Asp
1185 1190 1195 1200
Asn Leu Gln Ser Val Thr Leu Asp Ala Leu Gly Arg Val Thr Thr Leu
1205 1210 1215
Arg Phe Trp Gly Thr Glu Asn Gly Ile Ala Thr Gly Tyr Ser Asp Ala
1220 1225 1230
Thr Leu Ser Val Pro Asp Gly Ala Ala Ala Ala Leu Ala Leu Thr Ala
1235 1240 1245
Pro Leu Pro Val Ala Gln Cys Leu Val Tyr Val Thr Asp Ser Trp Gly
1250 1255 1260
Asp Asp Asp Asn Glu Lys Met Pro Pro His Val Val Val Leu Ala Thr
1265 1270 1275 1280
Asp Arg Tyr Asp Ser Asp Thr Gly Gln Gln Val Arg Gln Gln Val Thr
1285 1290 1295
Phe Ser Asp Gly Phe Gly Arg Glu Leu Gln Ser Ala Thr Arg Gln Ala
1300 1305 1310
Glu Gly Asn Ala Trp Gln Arg Gly Arg Asp Gly Lys Leu Val Thr Ala
1315 1320 1325
Ser Asp Gly Leu Pro Val Thr Val Ala Thr Asn Phe Arg Trp Ala Val
1330 1335 1340
Thr Gly Arg Ala Glu Tyr Asp Asn Lys Gly Leu Pro Val Arg Val Tyr
1345 1350 1355 1360
Gln Pro Tyr Phe Leu Asp Ser Trp Gln Tyr Val Ser Asp Asp Ser Ala
1365 1370 1375
Arg Gln Asp Leu Tyr Ala Asp Thr His Phe Tyr Asp Pro Thr Ala Arg
1380 1385 1390
Glu Trp Gln Val Ile Thr Ala Lys Gly Glu Arg Arg Gln Val Leu Tyr
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<213> Serratia entomophila

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Thr Pro Glu Glu Thr Asp Glu Arg Ile Thr Cys His Gln His Asp Glu
35 40 45
Arg Gly Ser Leu Ser Gln Ser Ala Asp Pro Arg Leu His Ala Ala Gly
50 55 60
Leu Thr Asn Phe Thr Tyr Leu Asn Ser Leu Thr Gly Thr Val Leu Gln
65 70 75 80
Ser Val Ser Ala Asp Ala Gly Thr Ser Leu Glu Leu Ser Asp Ala Ala
85 90 95
Gly Arg Ala Phe Leu Ala Val Thr Gly Ala Gly Thr Glu Asp Ala Val
100 105 110
Thr Arg Thr Trp Gln Tyr Glu Asp Asp Thr Leu Pro Gly Arg Pro Leu
115 120 125
Ser Ile Thr Glu Gln Val Thr Gly Glu Ala Ala Gln Ile Thr Glu Arg
130 135 140
Phe Val Tyr Ala Gly Asn Thr Asp Ala Glu Lys Ile Leu Asn Leu Ala
145 150 155 160
Gly Gln Cys Val Ser His Tyr Asp Thr Ala Gly Leu Val Gln Thr Asp
165 170 175

Ser Ile Ala Leu Ser Gly Val Pro Leu Ala Val Thr Arg Gln Leu Leu
180 185 190
Pro Asp Ala Ala Gly Ala Asn Trp Met Gly Glu Asp Ala Ser Ala Trp
195 200 205
Asn Asp Leu Leu Asp Gly Glu Thr Phe Phe Thr Gln Thr His Ala Asp
210 215 220
Ala Thr Gly Ala Val Leu Ser Ile Thr Asp Ala Lys Gly Asn Leu Gln
225 230 235 240
Arg Val Ala Tyr Asp Val Ala Gly Leu Leu Ser Gly Ser Trp Leu Thr
245 250 255
Leu Lys Asp Gly Thr Glu Gln Val Ile Val Ala Ser Leu Thr Tyr Ser
260 265 270
Ala Ala Gly Lys Lys Leu Arg Glu Glu His Gly Asn Gly Val Val Thr
275 280 285
Ser Tyr Ile Tyr Glu Pro Glu Thr Gln Arg Leu Thr Gly Ile Lys Thr
290 295 300
Glu Arg Pro Ser Gly His Val Ala Gly Ala Lys Val Leu Gln Asp Leu
305 310 315 320
Arg Tyr Thr Tyr Asp Pro Val Gly Asn Val Leu Ser Val Asn Asn Asp
325 330 335
Ala Glu Glu Thr Arg Phe Trp Arg Asn Gln Lys Val Val Pro Glu Asn
340 345 350
Thr Tyr Ile Tyr Asp Ser Leu Tyr Gln Leu Val Ser Ala Thr Gly Arg
355 360 365
Glu Met Ala Asn Ala Gly Gln Gln Gly Asn Asp Leu Pro Ser Ala Thr
370 375 380
Ala Pro Leu Pro Thr Asp Ser Ser Ala Tyr Thr Asn Tyr Thr Arg Thr
385 390 395 400
Tyr Arg Tyr Asp Arg Gly Gly Asn Leu Thr Gln Met Arg His Ser Ala
405 410 415
Pro Ala Thr Asn Asn Asn Tyr Thr Thr Asp Ile Thr Val Ser Asp Arg
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435 440 445
Asp Met Leu Phe Ser Ala Gly Gly His Gln Lys His Leu Gln Pro Gly
450 455 460
Gln Ala Leu Val Trp Thr Pro Arg Gly Glu Leu Gln Lys Val Thr Pro
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485 490 495
Ala Gly Ser Gln Arg Ile Ile Lys Thr Gly Thr Arg Gln Thr Gly Asn
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515 520 525
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530 535 540
Val Gly Glu Ala Gly Arg Ala Gln Val Arg Val Leu His Trp Glu Ile
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Gly Lys Pro Asp Asp Leu Asp Glu Asp Ser Val Arg Tyr Ser Tyr Asp
565 570 575
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580 585 590
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595 600 605
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Lys Glu Arg Asp Ala Thr Gly Leu Asp Tyr Tyr Gly Tyr Arg Tyr Tyr
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Gln Pro Trp Ala Gly Arg Trp Leu Ser Thr Asp Pro Ala Gly Thr Val
645 650 655
Asp Gly Leu Asn Leu Phe Arg Met Val Arg Asn Asn Pro Val Thr Leu
660 665 670
Phe Asp Ser Asn Gly Arg Ile Ser Thr Gly Gln Glu Ala Arg Arg Leu
675 680 685
Val Gly Glu Ala Phe Val His Pro Leu His Met Pro Val Phe Glu Arg
690 695 700
Ile Ser Val Glu Arg Lys Ile Ser Met Ser Val Arg Glu Ala Gly Ile

705 710 715 720
Tyr Thr Ile Ser Ala Leu Gly Glu Gly Ala Ala Ala Lys Gly His Asn
725 730 735
Ile Leu Glu Lys Thr Ile Lys Pro Gly Ser Leu Lys Ala Ile Tyr Gly
740 745 750
Asp Lys Ala Glu Ser Ile Leu Gly Leu Ala Lys Arg Ser Gly Leu Val
755 760 765
Gly Arg Val Gly Gln Trp Asp Ala Ser Gly Val Arg Gly Ile Tyr Ala
770 775 780
His Asn Arg Pro Gly Gly Glu Asp Leu Val Tyr Pro Val Ser Leu Gln
785 790 795 800
Asn Thr Ser Ala Asn Glu Ile Val Asn Ala Trp Ile Lys Phe Lys Ile
805 810 815
Ile Thr Pro Tyr Thr Gly Asp Tyr Asp Met His Asp Ile Ile Lys Phe
820 825 830
Ser Asp Gly Lys Gly His Val Pro Thr Ala Glu Ser Ser Glu Glu Arg
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Gly Val Lys Asp Leu Ile Asn Lys Gly Val Ala Glu Val Asp Pro Ser
850 855 860
Arg Pro Phe Glu Tyr Thr Ala Met Asn Val Ile Arg His Gly Pro Gln
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Val Asn Phe Val Pro Tyr Met Trp Glu His Glu His Asp Lys Val Val
885 890 895
Asn Asp Asn Gly Tyr Leu Gly Val Val Ala Ser Pro Gly Pro Phe Pro
900 905 910
Val Ala Met Val His Gln Gly Glu Trp Thr Val Phe Asp Asn Ser Glu
915 920 925
Glu Leu Phe Asn Phe Tyr Lys Ser Thr Asn Thr Pro Leu Pro Glu His
930 935 940
Trp Ser Gln Asp Phe Met Asp Arg Gly Lys Gly Ile Val Ala Thr Pro
945 950 955 960
Arg His Ala Glu Leu Leu Asp Lys Arg Arg Val Met Tyr
965 970

10/070489
PCT/NZ00/00174
Rec'd PCT/PTO 01 MAR 2002

SEQUENCE LISTING

(1) GENERAL INFORMATION

(i) APPLICANT: Glare, Travis T
Hurst, Mark R H
Jackson, Trevor A

(ii) TITLE OF INVENTION: Insecticidal nucleotide sequences

(iii) NUMBER OF SEQUENCES: 6

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: A J Park & Son
(B) STREET: Huddart Parker Building, Post Office Square
(C) CITY: Wellington
(D) COUNTRY: New Zealand

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18937 nucleotides	(A) LENGTH: 5118 amino acids
(B) TYPE: nucleotide	(B) TYPE: amino acid
(C) STRANDEDNESS: single	(C) STRANDEDNESS:
(D) TOPOLOGY: Linear	(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: DNA (ii) MOLECULE TYPE: PROTEIN

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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Tyr Gly His Thr Ala Gly Val Thr Lys Gly Asp Ile Ile Thr Val Asp
35 40 45

gaa gcc cag acg atg ctg aca aac gat att acc gta ttt gaa cgg gcg 2051
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50 55 60

gtc agt cag gcc gtc gcg gtt cct ctg aat cag tcg caa tac gat gcc 2099
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65 70 75 80

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Leu Val Ser Leu Val Phe Asn Ile Gly Gln Gly Asn Phe Lys Arg Ser
85 90 95

acc ttg ttg aaa aaa ctc aac aaa cag gac tat gtc ggc gcc ggg aac 2195
Thr Leu Leu Lys Lys Leu Asn Lys Gln Asp Tyr Val Gly Ala Gly Asn
100 105 110

gag ttt tta cgc tgg acc cgg gcc aat ggg aag gtc ctt ccc gga ctg 2243

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Ile Arg Arg Arg Glu Ala Glu Arg Val Leu Phe Glu Lys Leu Gly Ala
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145

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           Met Arg Gln Asp Ile Met Tyr Asn Ile Asp Asp Ile Leu
                        150                 155

gag aaa gtg aat gct cca cga gca cgc ctg tca gaa gaa aac gat aca    2500
Glu Lys Val Asn Ala Pro Arg Ala Arg Leu Ser Glu Glu Asn Asp Thr
160                 165                 170                 175

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Ala Val Thr Leu Thr Asp Leu Phe Ser Arg Ser Phe Pro Glu Val Lys
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Lys Ile Thr Gly Asp Ser Leu Ser Trp Gly Glu Val Cys Tyr Leu Tyr
            195                 200                 205

agt cag gcg cag cac gaa cag aaa gaa aac cgg ctc acc gaa tcc cgt    2644
Ser Gln Ala Gln His Glu Gln Lys Glu Asn Arg Leu Thr Glu Ser Arg
        210                 215                 220

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Ile Leu Ala Arg Ala Asn Pro Leu Leu Val Asn Ala Val Arg Leu Gly
    225                 230                 235

ata cgg cag gca gcc ggc agt cgc agc tat gat gac tgg ttt ggc tcc    2740
Ile Arg Gln Ala Ala Gly Ser Arg Ser Tyr Asp Asp Trp Phe Gly Ser
240                 245                 250                 255

cgc gca gac cgt ttc gcc cgc ccc ggc tcg gtg gcc tcc atg ttc tca    2788
Arg Ala Asp Arg Phe Ala Arg Pro Gly Ser Val Ala Ser Met Phe Ser
                260                 265                 270

ccg gcg gcg tat ctg acc gag ctg tac cgt gag gcg aag gac ctg cat    2836
Pro Ala Ala Tyr Leu Thr Glu Leu Tyr Arg Glu Ala Lys Asp Leu His
            275                 280                 285

ccg gac acc tcg ctg ttc cgg ctg gac atc cgg cgt ccc gac ctg gcg    2884
Pro Asp Thr Ser Leu Phe Arg Leu Asp Ile Arg Arg Pro Asp Leu Ala
        290                 295                 300

gcg ctg gcc ctt agc cag aat aat atg gac gac gag ctc tcc acc ctg    2932
Ala Leu Ala Leu Ser Gln Asn Asn Met Asp Asp Glu Leu Ser Thr Leu
    305                 310                 315

agc ctg tcc aat gag cta ctg tat cgc ggt atc ggg gca gcg gaa ggg    2980
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Ser Leu Ser Asn Glu Leu Leu Tyr Arg Gly Ile Gly Ala Ala Glu Gly
320                 325                 330                 335

ctt gac gac gac agc gtc agg gag ctg ctc gcc ggg tat cgc ctg acc     3028
Leu Asp Asp Asp Ser Val Arg Glu Leu Leu Ala Gly Tyr Arg Leu Thr
                340                 345                 350

ggc ctg acc ccc tat cac tgg gcg tac gag gcg gcc cgc caa gcc att     3076
Gly Leu Thr Pro Tyr His Trp Ala Tyr Glu Ala Ala Arg Gln Ala Ile
            355                 360                 365

ctg gtg cag gac ccg acg ctg atg ggg ttc agc cgt aat ccg gat gtg     3124
Leu Val Gln Asp Pro Thr Leu Met Gly Phe Ser Arg Asn Pro Asp Val
        370                 375                 380

gcg cag ctt atg gac cct gcc tcc atg ctg gcc att gaa gcc gat att     3172
Ala Gln Leu Met Asp Pro Ala Ser Met Leu Ala Ile Glu Ala Asp Ile
    385                 390                 395

tca ccg gag ctg tat cag ata ctg gcc gaa gaa att acg aca gac agt     3220
Ser Pro Glu Leu Tyr Gln Ile Leu Ala Glu Glu Ile Thr Thr Asp Ser
400                 405                 410                 415

tac gaa gca ctc tgg agt aag aat ttt ggt gat atg cct ccc tcc tca     3268
Tyr Glu Ala Leu Trp Ser Lys Asn Phe Gly Asp Met Pro Pro Ser Ser
                420                 425                 430

ctg tta tct tat gat gca ctt gca aca ttt tat gat ctt gat tac gat     3316
Leu Leu Ser Tyr Asp Ala Leu Ala Thr Phe Tyr Asp Leu Asp Tyr Asp
            435                 440                 445

gag cta act tcg tta ttg tca tta agg ctg gac ttt tca aat cca aac     3364
Glu Leu Thr Ser Leu Leu Ser Leu Arg Leu Asp Phe Ser Asn Pro Asn
        450                 455                 460

aat gaa tac tac att aat agt caa tta agt gtc gta act ctg aat gaa     3412
Asn Glu Tyr Tyr Ile Asn Ser Gln Leu Ser Val Val Thr Leu Asn Glu
    465                 470                 475

agc act ggt tta ata act ata cat cat tat tta aga acg cta ggc gga     3460
Ser Thr Gly Leu Ile Thr Ile His His Tyr Leu Arg Thr Leu Gly Gly
480                 485                 490                 495

gac tca cag cag att aac cct gag ctt ata cct tat ggg gat gga aca     3508
Asp Ser Gln Gln Ile Asn Pro Glu Leu Ile Pro Tyr Gly Asp Gly Thr
                500                 505                 510

tat ctt tat aat ttc agc gtg gtg tca acg ata tca gag gat agt ttc     3556
Tyr Leu Tyr Asn Phe Ser Val Val Ser Thr Ile Ser Glu Asp Ser Phe
            515                 520                 525

aaa cta ggg tcg tta ggt tct aac agt agc aat ctt tac tct ggg gat     3604
Lys Leu Gly Ser Leu Gly Ser Asn Ser Ser Asn Leu Tyr Ser Gly Asp
        530                 535                 540

tat cag ctt caa aaa ggg gtt cgc tat agc att cct gtt gaa ata gat     3652
Tyr Gln Leu Gln Lys Gly Val Arg Tyr Ser Ile Pro Val Glu Ile Asp
    545                 550                 555
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gaa gga aag tta aat gat ggg atc aca ata gga ttg agt agg aaa ggg 3700
Glu Gly Lys Leu Asn Asp Gly Ile Thr Ile Gly Leu Ser Arg Lys Gly
560 565 570 575

ggg gga tat tac tca aca gta aac ttc act ctg att gaa tat gat cct 3748
Gly Gly Tyr Tyr Ser Thr Val Asn Phe Thr Leu Ile Glu Tyr Asp Pro
580 585 590

gcg ata ttc att ctt aaa tta aat aaa gtt atc cgc cta tac aag gcc 3796
Ala Ile Phe Ile Leu Lys Leu Asn Lys Val Ile Arg Leu Tyr Lys Ala
595 600 605

acg ggc atg acc acg gcg gaa ata tat caa atc acc aat att ctt aat 3844
Thr Gly Met Thr Thr Ala Glu Ile Tyr Gln Ile Thr Asn Ile Leu Asn
610 615 620

aac ggt ctc acc att gac cat gcg gtc ctg agt aaa atc ttc ctg gtc 3892
Asn Gly Leu Thr Ile Asp His Ala Val Leu Ser Lys Ile Phe Leu Val
625 630 635

cgt tac ctg atg cgt cac tat cag ctt gat gtg gcc cgg tca ctg ata 3940
Arg Tyr Leu Met Arg His Tyr Gln Leu Asp Val Ala Arg Ser Leu Ile
640 645 650 655

ttg tgc aac gga acc atc agt gac cag gcg ttc agc ggc gaa acc ggc 3988
Leu Cys Asn Gly Thr Ile Ser Asp Gln Ala Phe Ser Gly Glu Thr Gly
660 665 670

ctg ttc acc acg ctg ttc aac acc cca ccg ctg aac ggc cag ctg ttt 4036
Leu Phe Thr Thr Leu Phe Asn Thr Pro Pro Leu Asn Gly Gln Leu Phe
675 680 685

tct gca gat gat acc ccc ctc gac tta cgc tct gaa gca ccg gag gat 4084
Ser Ala Asp Asp Thr Pro Leu Asp Leu Arg Ser Glu Ala Pro Glu Asp
690 695 700

gct ttc cgt ctc agc gta ctg aaa cgc gca ttt aac atc agc gcc tcg 4132
Ala Phe Arg Leu Ser Val Leu Lys Arg Ala Phe Asn Ile Ser Ala Ser
705 710 715

ggg ctt tcc acg ctc tgg cag ttg gcc agc ggt gac agc agc gct ggg 4180
Gly Leu Ser Thr Leu Trp Gln Leu Ala Ser Gly Asp Ser Ser Ala Gly
720 725 730 735

ttt agc tgc tct gct gac aat atc gcc gca ctc tac cga gtg aaa ctc 4228
Phe Ser Cys Ser Ala Asp Asn Ile Ala Ala Leu Tyr Arg Val Lys Leu
740 745 750

ctg gct gac atc cac gac cta tcc gct ggt gag ctg tca atg ttg ctg 4276
Leu Ala Asp Ile His Asp Leu Ser Ala Gly Glu Leu Ser Met Leu Leu
755 760 765

tcc gtc tcc cct ttc agc ggg gtg gcc gcc ggc tcg ctg tcc gat aat 4324
Ser Val Ser Pro Phe Ser Gly Val Ala Ala Gly Ser Leu Ser Asp Asn
770 775 780

gag ctg acg cag ttt ctg tac cag acc acc acc tgg ctc acg gag cag 4372

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Glu Leu Thr Gln Phe Leu Tyr Gln Thr Thr Thr Trp Leu Thr Glu Gln
    785                 790                 795

ggc tgg acg gtc agc gat gtg ttc ctg atg ctg acg acg cag tac ggt    4420
Gly Trp Thr Val Ser Asp Val Phe Leu Met Leu Thr Thr Gln Tyr Gly
800                 805                 810                 815

acc ctg ctg acc ccc gac att gag aac ctg ctc gct tcc ctg cgc aac    4468
Thr Leu Leu Thr Pro Asp Ile Glu Asn Leu Leu Ala Ser Leu Arg Asn
                820                 825                 830

gga ctg tcg ggc cgt gag ctg ttc ccg gaa acg ctc ccc ggc gat ggc    4516
Gly Leu Ser Gly Arg Glu Leu Phe Pro Glu Thr Leu Pro Gly Asp Gly
            835                 840                 845

gct ccc ttt att gcc gcc gcc atg cag ctg gac gcc acg gat acg gcg    4564
Ala Pro Phe Ile Ala Ala Ala Met Gln Leu Asp Ala Thr Asp Thr Ala
        850                 855                 860

aag gcg atg ctg act tgg gcg gac cag ttg aag cca gag ggg ctg acg    4612
Lys Ala Met Leu Thr Trp Ala Asp Gln Leu Lys Pro Glu Gly Leu Thr
    865                 870                 875

ctg acg gaa ttt att ctt ttg gtg atg aat gcc gcc cca aat gac gag    4660
Leu Thr Glu Phe Ile Leu Leu Val Met Asn Ala Ala Pro Asn Asp Glu
880                 885                 890                 895

cag gcg ggc cag atg gca ggg ttc tgc caa gcc ctg tgg caa ctg gca    4708
Gln Ala Gly Gln Met Ala Gly Phe Cys Gln Ala Leu Trp Gln Leu Ala
                900                 905                 910

ctg atc atc cgc agc acc ggc ctc agc acg cgc gag ctg acg ctg ctg    4756
Leu Ile Ile Arg Ser Thr Gly Leu Ser Thr Arg Glu Leu Thr Leu Leu
            915                 920                 925

gtc agc cag ccg gga cgc ttc cgc aca gga tgg cac cat ctg ccc cat    4804
Val Ser Gln Pro Gly Arg Phe Arg Thr Gly Trp His His Leu Pro His
        930                 935                 940

gac ctg ccg gcg ctt cgc gac att acg cgt ttt cat gcc gtc gtt aac    4852
Asp Leu Pro Ala Leu Arg Asp Ile Thr Arg Phe His Ala Val Val Asn
    945                 950                 955

cgc agc ggc agc cat gcc ggg gag gtc ctg acc gca ctt gag acc gga    4900
Arg Ser Gly Ser His Ala Gly Glu Val Leu Thr Ala Leu Glu Thr Gly
960                 965                 970                 975

gaa ctg tcg tca gcc ctg ctg gcc cgg gcc ctg tca cag aat gag cag    4948
Glu Leu Ser Ser Ala Leu Leu Ala Arg Ala Leu Ser Gln Asn Glu Gln
                980                 985                 990

gat gtg acc ggc gcc ttg gcg cag gtg agg ggg gcc ggt gaa cag gac    4996
Asp Val Thr Gly Ala Leu Ala Gln Val Arg Gly Ala Gly Glu Gln Asp
            995                 1000                1005

aac agc gtg ttc acc tcc tgg gaa gag gtg gac cag gct gag cag tgg    5044
Asn Ser Val Phe Thr Ser Trp Glu Glu Val Asp Gln Ala Glu Gln Trp
        1010                1015                1020
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ctg gac atg agt gag acc ctg tcc att acg cca tcc ggt ctg gct agc   5092
Leu Asp Met Ser Glu Thr Leu Ser Ile Thr Pro Ser Gly Leu Ala Ser
   1025                1030                1035

ctg att gcc ctg aag tac atc aat gtg tcc gat gac agt gca ccg ttg   5140
Leu Ile Ala Leu Lys Tyr Ile Asn Val Ser Asp Asp Ser Ala Pro Leu
1040                1045                1050                1055

tac agc cag tgg cag gtg gta tcc ggt ctg ctg cag gcc ggg ctg aaa   5188
Tyr Ser Gln Trp Gln Val Val Ser Gly Leu Leu Gln Ala Gly Leu Lys
               1060                1065                1070

agc agc cag agc tcg gcg ctg cac gat tat ctg gag gag ggg acc agc   5236
Ser Ser Gln Ser Ser Ala Leu His Asp Tyr Leu Glu Glu Gly Thr Ser
           1075                1080                1085

agc gcc ctt tgt gcg tat tat ctg cgt aat ctg gca ccg aac atg gta   5284
Ser Ala Leu Cys Ala Tyr Tyr Leu Arg Asn Leu Ala Pro Asn Met Val
       1090                1095                1100

tcc ggg cgc gat gac ctc ttc ggg tat ctg ctg ctg gat aat cag gtg   5332
Ser Gly Arg Asp Asp Leu Phe Gly Tyr Leu Leu Leu Asp Asn Gln Val
   1105                1110                1115

tca gcc aag gta aaa acc acc cgc att gcg gag gcc atc gcc ggc ata   5380
Ser Ala Lys Val Lys Thr Thr Arg Ile Ala Glu Ala Ile Ala Gly Ile
1120                1125                1130                1135

cgg ctg tat atc aac cgg gcc ctt aac gga ata gaa ctc agc gcc atg   5428
Arg Leu Tyr Ile Asn Arg Ala Leu Asn Gly Ile Glu Leu Ser Ala Met
               1140                1145                1150

gca gag gtg agg ggg cgt cag ttt ttc act gac tgg gat acg ttc aac   5476
Ala Glu Val Arg Gly Arg Gln Phe Phe Thr Asp Trp Asp Thr Phe Asn
           1155                1160                1165

aaa cgt tac agc acc tgg gcg ggc gtc tca gag ctg gtt tac tat ccg   5524
Lys Arg Tyr Ser Thr Trp Ala Gly Val Ser Glu Leu Val Tyr Tyr Pro
       1170                1175                1180

gaa aac tac ctc gac ccg acg gtc cgt atc ggg cag acc ggc atg atg   5572
Glu Asn Tyr Leu Asp Pro Thr Val Arg Ile Gly Gln Thr Gly Met Met
   1185                1190                1195

gac acc ctg ctg cag tct gtc agc cag agc agt atc aac cgc gat acc   5620
Asp Thr Leu Leu Gln Ser Val Ser Gln Ser Ser Ile Asn Arg Asp Thr
1200                1205                1210                1215

gtg gag gat gcc ttt aaa acc tat ctg acc acg ttt gag cag att gcc   5668
Val Glu Asp Ala Phe Lys Thr Tyr Leu Thr Thr Phe Glu Gln Ile Ala
               1220                1225                1230

aat ctg aac act gtc agc gga tat cac gat aac gcc agc atg acg cag   5716
Asn Leu Asn Thr Val Ser Gly Tyr His Asp Asn Ala Ser Met Thr Gln
           1235                1240                1245

ggg act aca tgg tat gtg ggt cgc agc atc aca gat cag act aac tgg   5764
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Gly Thr Thr Trp Tyr Val Gly Arg Ser Ile Thr Asp Gln Thr Asn Trp
      1250                1255                1260

tac tgg cgc agc gcc aac cac agc aaa atc caa gac tca atg atg ccc    5812
Tyr Trp Arg Ser Ala Asn His Ser Lys Ile Gln Asp Ser Met Met Pro
   1265                1270                1275

gcg aat gcc tgg acc gga tgg aca aaa att aac tgc gga atg aat ccg    5860
Ala Asn Ala Trp Thr Gly Trp Thr Lys Ile Asn Cys Gly Met Asn Pro
1280                1285                1290                1295

tgg tca gat ctt gtg tgc tcg gtg ttt ttc aac agt cgc ctt tat gtc    5908
Trp Ser Asp Leu Val Cys Ser Val Phe Phe Asn Ser Arg Leu Tyr Val
                1300                1305                1310

gtc tgg gtc gaa gag aat cag tct gct gat acg gag gca gag agc acg    5956
Val Trp Val Glu Glu Asn Gln Ser Ala Asp Thr Glu Ala Glu Ser Thr
            1315                1320                1325

aca acc acg cag cag agc tac acg ctg aaa ctg tcg ttc cgg cgc tac    6004
Thr Thr Thr Gln Gln Ser Tyr Thr Leu Lys Leu Ser Phe Arg Arg Tyr
        1330                1335                1340

gac ggt aca tgg agt tcc ccg gtg tcg ttc gac att acc ggc aac atc    6052
Asp Gly Thr Trp Ser Ser Pro Val Ser Phe Asp Ile Thr Gly Asn Ile
    1345                1350                1355

gca ttt ccg gaa acg cag ggc atg cat gtg acc tgt aat ccc ctg act    6100
Ala Phe Pro Glu Thr Gln Gly Met His Val Thr Cys Asn Pro Leu Thr
1360                1365                1370                1375

gag cag ctc tat tgc gcg ttt tac tcc gtc acc agc aag ccg gac ttt    6148
Glu Gln Leu Tyr Cys Ala Phe Tyr Ser Val Thr Ser Lys Pro Asp Phe
                1380                1385                1390

gat aac gct cag ctg att tct gtg gat aat gat atg acg cta aat gtc    6196
Asp Asn Ala Gln Leu Ile Ser Val Asp Asn Asp Met Thr Leu Asn Val
            1395                1400                1405

atc tca gat ata ggg att ttt aag agc gtc agt cac gaa ttt aat acg    6244
Ile Ser Asp Ile Gly Ile Phe Lys Ser Val Ser His Glu Phe Asn Thr
        1410                1415                1420

agc act gag aaa ttt att aat aat gtt ttt tca gac cct tcc gct aat    6292
Ser Thr Glu Lys Phe Ile Asn Asn Val Phe Ser Asp Pro Ser Ala Asn
    1425                1430                1435

tat ttt gtc agt gca acg agt tta att gat gat gtt atc cac agc gat    6340
Tyr Phe Val Ser Ala Thr Ser Leu Ile Asp Asp Val Ile His Ser Asp
1440                1445                1450                1455

ttc tca ctc ctt aat tct aaa act aca agt act gtt ttt act aat gaa    6388
Phe Ser Leu Leu Asn Ser Lys Thr Thr Ser Thr Val Phe Thr Asn Glu
                1460                1465                1470

gat tcc tct ctt ttg acg cca gag ctt cat att aca gca aat gtt tcg    6436
Asp Ser Ser Leu Leu Thr Pro Glu Leu His Ile Thr Ala Asn Val Ser
            1475                1480                1485
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tgt ttt gtt agt act gct ggc atc gcc act caa tct acc ata gaa aaa 6484
Cys Phe Val Ser Thr Ala Gly Ile Ala Thr Gln Ser Thr Ile Glu Lys
1490 1495 1500

ttc gtt cag gca ggg ata gaa ttt gag gaa att aat ttt tat gca ggc 6532
Phe Val Gln Ala Gly Ile Glu Phe Glu Glu Ile Asn Phe Tyr Ala Gly
1505 1510 1515

cag gcc gcc ggc gga ttt gac gga ttt gtg gga gtg gat gtt tct aat 6580
Gln Ala Ala Gly Gly Phe Asp Gly Phe Val Gly Val Asp Val Ser Asn
1520 1525 1530 1535

tca aaa gta tac cag gtc gga aaa gaa gca gtt ggt gtc act gta aaa 6628
Ser Lys Val Tyr Gln Val Gly Lys Glu Ala Val Gly Val Thr Val Lys
1540 1545 1550

tct tat tcc gtc act ggc gtt agt ggt tct gtt gag tta ttt att gat 6676
Ser Tyr Ser Val Thr Gly Val Ser Gly Ser Val Glu Leu Phe Ile Asp
1555 1560 1565

tca tca aat aaa tac ttc agc gga att ttg tca gat aaa atg ata acc 6724
Ser Ser Asn Lys Tyr Phe Ser Gly Ile Leu Ser Asp Lys Met Ile Thr
1570 1575 1580

gct tta att agc ggc agt aca tca aaa gtt aat tac gtg tcg tct att 6772
Ala Leu Ile Ser Gly Ser Thr Ser Lys Val Asn Tyr Val Ser Ser Ile
1585 1590 1595

ggc tct caa gat ttt tgg agt gta aag tcg ctc atg ccg gca ctt cag 6820
Gly Ser Gln Asp Phe Trp Ser Val Lys Ser Leu Met Pro Ala Leu Gln
1600 1605 1610 1615

ata tat gaa tta atc gat gat atc ata ctg aca tcc ggc gta aat ggg 6868
Ile Tyr Glu Leu Ile Asp Asp Ile Ile Leu Thr Ser Gly Val Asn Gly
1620 1625 1630

act gaa att aaa tcc tgg cct tcc gct gaa tgg tat aat gat aag ctg 6916
Thr Glu Ile Lys Ser Trp Pro Ser Ala Glu Trp Tyr Asn Asp Lys Leu
1635 1640 1645

agt ctg caa tcc ggg aat aat ctt ttc aac acc aaa tcg ctg agt ttt 6964
Ser Leu Gln Ser Gly Asn Asn Leu Phe Asn Thr Lys Ser Leu Ser Phe
1650 1655 1660

acc gtt aat acc agt gat att gtt gaa gat gag ttt gac gtg acg ttt 7012
Thr Val Asn Thr Ser Asp Ile Val Glu Asp Glu Phe Asp Val Thr Phe
1665 1670 1675

acg ttc acc gct gtc gat cag aat aac gtc gtg ctg gcc gcc cgg acg 7060
Thr Phe Thr Ala Val Asp Gln Asn Asn Val Val Leu Ala Ala Arg Thr
1680 1685 1690 1695

gcc ata tta acc gtc att cga aac att aat aat gac act tcc gtt atc 7108
Ala Ile Leu Thr Val Ile Arg Asn Ile Asn Asn Asp Thr Ser Val Ile
1700 1705 1710

gca tta cgt aaa aat acg cgt ggc gcg cag tat att cgt ttc act gcg 7156

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Ala Leu Arg Lys Asn Thr Arg Gly Ala Gln Tyr Ile Arg Phe Thr Ala
        1715                1720                1725

ggt aac gat gtg gcg ctt att cgc ctc aac acc ctc ttt gcc cgc caa    7204
Gly Asn Asp Val Ala Leu Ile Arg Leu Asn Thr Leu Phe Ala Arg Gln
     1730                1735                1740

ctg gtc gac cgg gcg aat acc ggg att gac acc att ctt tcc atg gag    7252
Leu Val Asp Arg Ala Asn Thr Gly Ile Asp Thr Ile Leu Ser Met Glu
  1745                1750                1755

acc cag agg ctt acc gaa ccc gcc ctg gaa gag ggg agt gat gtg ttt    7300
Thr Gln Arg Leu Thr Glu Pro Ala Leu Glu Glu Gly Ser Asp Val Phe
1760                1765                1770                1775

atg gac ttc tcc gga gcc aat gcc ctc tat ttc tgg gag ctg ttc tat    7348
Met Asp Phe Ser Gly Ala Asn Ala Leu Tyr Phe Trp Glu Leu Phe Tyr
                1780                1785                1790

tac acg ccg atg atg gtg ttc cag cgg ttg ttg cag gaa cag cac ttc    7396
Tyr Thr Pro Met Met Val Phe Gln Arg Leu Leu Gln Glu Gln His Phe
            1795                1800                1805

ccg gaa gcc acc cgc tgg ctg cag tat gtc tgg aac ccg gcc ggg cac    7444
Pro Glu Ala Thr Arg Trp Leu Gln Tyr Val Trp Asn Pro Ala Gly His
        1810                1815                1820

gtg gta aac ggg gtg ctg cag aat tac acc tgg aat gtc cgt ccg ctg    7492
Val Val Asn Gly Val Leu Gln Asn Tyr Thr Trp Asn Val Arg Pro Leu
    1825                1830                1835

gag gag gac acc ggc tgg aac gac tcg ccg ctg gac tcc att gac ccc    7540
Glu Glu Asp Thr Gly Trp Asn Asp Ser Pro Leu Asp Ser Ile Asp Pro
1840                1845                1850                1855

gat gca ata gcc cag tac gac ccc atg cat tac aag gtc gcc acc ttt    7588
Asp Ala Ile Ala Gln Tyr Asp Pro Met His Tyr Lys Val Ala Thr Phe
                1860                1865                1870

atg tcg tac ctc gac ctg ctg att gcc cgc ggt gat gcc gcc tac cgg    7636
Met Ser Tyr Leu Asp Leu Leu Ile Ala Arg Gly Asp Ala Ala Tyr Arg
            1875                1880                1885

ctg ctc gag cgg gac acc ctt aac gag gcc cgg atg tgg tac gtc cag    7684
Leu Leu Glu Arg Asp Thr Leu Asn Glu Ala Arg Met Trp Tyr Val Gln
        1890                1895                1900

gcc ctg aac ctt ctg ggc gac gag ccc tat att tcc ttt gac gcc gac    7732
Ala Leu Asn Leu Leu Gly Asp Glu Pro Tyr Ile Ser Phe Asp Ala Asp
    1905                1910                1915

tgg tcg gcg ttg acc ctg ggt gac gca gcc agc gag gtg acg cga cgc    7780
Trp Ser Ala Leu Thr Leu Gly Asp Ala Ala Ser Glu Val Thr Arg Arg
1920                1925                1930                1935

gat tac cag gag gcc ctg ctg gcc gtg cgc cgg ttg gtg ccc gct ccc    7828
Asp Tyr Gln Glu Ala Leu Leu Ala Val Arg Arg Leu Val Pro Ala Pro
                1940                1945                1950
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gag aca cgg acg gcg aat tcc ctg acg gca ctg ttc ctc ccg cag cag 7876
Glu Thr Arg Thr Ala Asn Ser Leu Thr Ala Leu Phe Leu Pro Gln Gln
1955 1960 1965

aac gag gtg ctc aaa ggc tac tgg caa acc ttg gca cag cgg ctc cat 7924
Asn Glu Val Leu Lys Gly Tyr Trp Gln Thr Leu Ala Gln Arg Leu His
1970 1975 1980

aac ctg cgc cac aac ctc tcc att gac ggc cag ccg ctt tcc ctg tcc 7972
Asn Leu Arg His Asn Leu Ser Ile Asp Gly Gln Pro Leu Ser Leu Ser
1985 1990 1995

gtc tac gcc acg ccg tcc gaa ccg tcc gcc ctg cag agt gcc gtc gtc 8020
Val Tyr Ala Thr Pro Ser Glu Pro Ser Ala Leu Gln Ser Ala Val Val
2000 2005 2010 2015

aac agc gcg cag ggt gct gca gca ctg ccg gcc gcg gtg atg ccg ctt 8068
Asn Ser Ala Gln Gly Ala Ala Ala Leu Pro Ala Ala Val Met Pro Leu
2020 2025 2030

tac agt ttc ccg gtc atg ctg gag aac gcc cgg ggg atg gtg agc ctg 8116
Tyr Ser Phe Pro Val Met Leu Glu Asn Ala Arg Gly Met Val Ser Leu
2035 2040 2045

ctg acc ggg ttc ggc aac aca ctg ctc ggt att acc gag cgt cag gat 8164
Leu Thr Gly Phe Gly Asn Thr Leu Leu Gly Ile Thr Glu Arg Gln Asp
2050 2055 2060

gcg gag gcg ctg gcc aaa ctg ctg cag acc cag ggc agt gaa ctg ata 8212
Ala Glu Ala Leu Ala Lys Leu Leu Gln Thr Gln Gly Ser Glu Leu Ile
2065 2070 2075

cgc cag ggc ctt cgc cag cag gat aac gtc ctc gag gaa atc gat gcg 8260
Arg Gln Gly Leu Arg Gln Gln Asp Asn Val Leu Glu Glu Ile Asp Ala
2080 2085 2090 2095

gat att gcc gcc ctg gag gag agc cgc cgc ggc gcg cag atg cgt ttt 8308
Asp Ile Ala Ala Leu Glu Glu Ser Arg Arg Gly Ala Gln Met Arg Phe
2100 2105 2110

gaa cgt tac aaa gtg ttg tac gag gcg gac gtc aac acc ggc gaa aaa 8356
Glu Arg Tyr Lys Val Leu Tyr Glu Ala Asp Val Asn Thr Gly Glu Lys
2115 2120 2125

cag gcc atg gac ttg tac ctc agt tcg tcc gtg ctg tcg gca tca acc 8404
Gln Ala Met Asp Leu Tyr Leu Ser Ser Ser Val Leu Ser Ala Ser Thr
2130 2135 2140

gcc gcg ctc ttt ttg gcc gag gcc gcg gcc gat atg ctg ccc aat att 8452
Ala Ala Leu Phe Leu Ala Glu Ala Ala Ala Asp Met Leu Pro Asn Ile
2145 2150 2155

tac ggg ctg gcc gtc ggg ggc tcc cgc tat ggg gca cta ttt aaa gcc 8500
Tyr Gly Leu Ala Val Gly Gly Ser Arg Tyr Gly Ala Leu Phe Lys Ala
2160 2165 2170 2175

acc gcc atc ggc atc cag gtg tcc tcc gat gcc acc cgc ata tca gcg 8548

Thr Ala Ile Gly Ile Gln Val Ser Ser Asp Ala Thr Arg Ile Ser Ala
2180 2185 2190

gac aaa atc agc cag tcg gaa gtg tac cgc cgt cgc cgg gag gag tgg 8596
Asp Lys Ile Ser Gln Ser Glu Val Tyr Arg Arg Arg Arg Glu Glu Trp
2195 2200 2205

gaa atc cag cgt gat agt gcg cag tct gac gtg gcg cag att gat gcc 8644
Glu Ile Gln Arg Asp Ser Ala Gln Ser Asp Val Ala Gln Ile Asp Ala
2210 2215 2220

cag ctg gcg gcc atg gca gtg cgc cgg gaa ggg gct gag ctg cag aaa 8692
Gln Leu Ala Ala Met Ala Val Arg Arg Glu Gly Ala Glu Leu Gln Lys
2225 2230 2235

act tac ctt gag acc cag cag acc cag gca cag gcg cag ttg gca ttc 8740
Thr Tyr Leu Glu Thr Gln Gln Thr Gln Ala Gln Ala Gln Leu Ala Phe
2240 2245 2250 2255

ctg cag agt aag ttc aac aat acg gct ctg tac agc tgg ctg cgg ggc 8788
Leu Gln Ser Lys Phe Asn Asn Thr Ala Leu Tyr Ser Trp Leu Arg Gly
2260 2265 2270

agg ttg tcc gcc att tat tac cag ttc tat gac ctg gca gta tcc cgc 8836
Arg Leu Ser Ala Ile Tyr Tyr Gln Phe Tyr Asp Leu Ala Val Ser Arg
2275 2280 2285

tgc ctg atg gcg caa cag gcc tgg cag tgg gat aaa ttc gag act agg 8884
Cys Leu Met Ala Gln Gln Ala Trp Gln Trp Asp Lys Phe Glu Thr Arg
2290 2295 2300

tcg ttt atc cag ccg ggg gcc tgg atg ggg gca aat gcc ggt ctg ctg 8932
Ser Phe Ile Gln Pro Gly Ala Trp Met Gly Ala Asn Ala Gly Leu Leu
2305 2310 2315

gcc ggg gaa acc ctg atg ctg aat ctg gcg cag atg gag cag gcc tgg 8980
Ala Gly Glu Thr Leu Met Leu Asn Leu Ala Gln Met Glu Gln Ala Trp
2320 2325 2330 2335

ctg acg ggg gat gag cgg gca ata gag gtg acg cgg acg gtc tgc ctg 9028
Leu Thr Gly Asp Glu Arg Ala Ile Glu Val Thr Arg Thr Val Cys Leu
2340 2345 2350

tcg gag gtc tat acc agc ctc gcg gag gat gcg gca ttc tct ctg gcc 9076
Ser Glu Val Tyr Thr Ser Leu Ala Glu Asp Ala Ala Phe Ser Leu Ala
2355 2360 2365

gac aag gtg gtg gaa ctg gtc agt aac ggt tcg ggc agt gcg ggt acg 9124
Asp Lys Val Val Glu Leu Val Ser Asn Gly Ser Gly Ser Ala Gly Thr
2370 2375 2380

aaa agc aac gga tta cag atg gat caa cag caa ctc gag gcc acc ctg 9172
Lys Ser Asn Gly Leu Gln Met Asp Gln Gln Gln Leu Glu Ala Thr Leu
2385 2390 2395

aaa ctg gct gac ctc ggt atc ggc aac gat tac ccg gtc tcc ctt ggc 9220
Lys Leu Ala Asp Leu Gly Ile Gly Asn Asp Tyr Pro Val Ser Leu Gly
2400 2405 2410 2415

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acc atg agg cgc atc aaa caa ata agc gtc acg ctc ccg gcg ctg gtc    9268
Thr Met Arg Arg Ile Lys Gln Ile Ser Val Thr Leu Pro Ala Leu Val
                2420                2425                2430

ggc ccc tat cag gac gtc cgt gcg gtt ctc agc tac ggc gga agt atg    9316
Gly Pro Tyr Gln Asp Val Arg Ala Val Leu Ser Tyr Gly Gly Ser Met
            2435                2440                2445

gtc atg ccc cgg ggt tgc agc gcg ctg gcg gtc tca cac gga atg aac    9364
Val Met Pro Arg Gly Cys Ser Ala Leu Ala Val Ser His Gly Met Asn
        2450                2455                2460

gac agc ggc caa ttc caa ctg gat ttc aat gac ccg cgt tac ctg ccg    9412
Asp Ser Gly Gln Phe Gln Leu Asp Phe Asn Asp Pro Arg Tyr Leu Pro
    2465                2470                2475

ttt gaa gga ctt cca gtt gat gac aca ggg acc ctg aca ctg agc ttc    9460
Phe Glu Gly Leu Pro Val Asp Asp Thr Gly Thr Leu Thr Leu Ser Phe
2480                2485                2490                2495

ccg gat gct gac ggc aaa caa cag gcg atg ctc ctc agt ctg agc gac    9508
Pro Asp Ala Asp Gly Lys Gln Gln Ala Met Leu Leu Ser Leu Ser Asp
                2500                2505                2510

atc atc ctg cat atc cgt tac acc att atc agc tga tag gtatcaacat     9557
Ile Ile Leu His Ile Arg Tyr Thr Ile Ile Ser
            2515                2520

agcgcaggcc cccgaacgag ggcctgcgag gagactgagc atg caa aat cat caa    9612
                                            Met Gln Asn His Gln
                                            2525

gac atg gcc att act gcc ccc acg ttg cct tcc ggg ggc ggt gcg gtc    9660
Asp Met Ala Ile Thr Ala Pro Thr Leu Pro Ser Gly Gly Gly Ala Val
2530                2535                2540                2545

acc ggg ctc aag ggt gat atc gcg gcg gca ggg ccg gat ggt gcg gcg    9708
Thr Gly Leu Lys Gly Asp Ile Ala Ala Ala Gly Pro Asp Gly Ala Ala
                2550                2555                2560

acc ctg agt att ccc ttg ccg gtt agc ccc ggt cgg ggt tac gcc ccc    9756
Thr Leu Ser Ile Pro Leu Pro Val Ser Pro Gly Arg Gly Tyr Ala Pro
            2565                2570                2575

act ggg gca ctt aat tat cac agc cgg tcg ggg aac ggc ccc ttt ggc    9804
Thr Gly Ala Leu Asn Tyr His Ser Arg Ser Gly Asn Gly Pro Phe Gly
        2580                2585                2590

att ggc tgg ggt atc ggc ggt gct gct gtc cag cgt cgt acg cgc aac    9852
Ile Gly Trp Gly Ile Gly Gly Ala Ala Val Gln Arg Arg Thr Arg Asn
    2595                2600                2605

gga gca cct acc tac gat gat act gat gaa ttc acc ggt ccg gac ggt    9900
Gly Ala Pro Thr Tyr Asp Asp Thr Asp Glu Phe Thr Gly Pro Asp Gly
2610                2615                2620                2625

gag gtg ctg gtg ccg gca ctc acg gct gct ggc acc caa gaa gca cgg    9948
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Glu Val Leu Val Pro Ala Leu Thr Ala Ala Gly Thr Gln Glu Ala Arg
2630 2635 2640

cag gcc acc tca cta ctg ggg ata aac cca ggc gga agc ttc aac gtt 9996
Gln Ala Thr Ser Leu Leu Gly Ile Asn Pro Gly Gly Ser Phe Asn Val
2645 2650 2655

cag gtt tac cgt tca cgt acg gag ggt agt ctc agc cgc ctt gag cgt 10044
Gln Val Tyr Arg Ser Arg Thr Glu Gly Ser Leu Ser Arg Leu Glu Arg
2660 2665 2670

tgg ctg ccc gcc gac gag aca gaa acg gaa ttt tgg gtg tta tat acc 10092
Trp Leu Pro Ala Asp Glu Thr Glu Thr Glu Phe Trp Val Leu Tyr Thr
2675 2680 2685

cct gac gga cag gtg gct ctg ctg ggc cga aat gcg cag gct cgc atc 10140
Pro Asp Gly Gln Val Ala Leu Leu Gly Arg Asn Ala Gln Ala Arg Ile
2690 2695 2700 2705

agc aac ccc aca gcc cca aca cag acg gcg gtt tgg ctg atg gag tcc 10188
Ser Asn Pro Thr Ala Pro Thr Gln Thr Ala Val Trp Leu Met Glu Ser
2710 2715 2720

tcg gta tca ctt acc ggc gaa cag atg tat tac caa tac cgt gcg gaa 10236
Ser Val Ser Leu Thr Gly Glu Gln Met Tyr Tyr Gln Tyr Arg Ala Glu
2725 2730 2735

gat gat gac ggt tgt gac gag gcg gag cgc gac gcg cac ccg cag gcc 10284
Asp Asp Asp Gly Cys Asp Glu Ala Glu Arg Asp Ala His Pro Gln Ala
2740 2745 2750

ggc gcc caa cgt tat ccg gtg gcg gtc tgg tat ggt aac cgt cag gcg 10332
Gly Ala Gln Arg Tyr Pro Val Ala Val Trp Tyr Gly Asn Arg Gln Ala
2755 2760 2765

gct cgg acg cta ccg gcg ctg gtg tcg aca cca tca atg gat agc tgg 10380
Ala Arg Thr Leu Pro Ala Leu Val Ser Thr Pro Ser Met Asp Ser Trp
2770 2775 2780 2785

ctg ttt atc ctg gtg ttt gat tat ggt gag cgt agc tcg gtg ctg tct 10428
Leu Phe Ile Leu Val Phe Asp Tyr Gly Glu Arg Ser Ser Val Leu Ser
2790 2795 2800

gaa gcg ccg gcc tgg caa aca cca gga agt ggg gag tgg ctg tgt cgt 10476
Glu Ala Pro Ala Trp Gln Thr Pro Gly Ser Gly Glu Trp Leu Cys Arg
2805 2810 2815

cag gat tgt ttt tcc ggg tat gag ttt ggt ttt aac ctg cgg act cgc 10524
Gln Asp Cys Phe Ser Gly Tyr Glu Phe Gly Phe Asn Leu Arg Thr Arg
2820 2825 2830

cgc ctg tgc cgt cag gtt ttg atg ttc cat tac cta ggt gtt ctg gcg 10572
Arg Leu Cys Arg Gln Val Leu Met Phe His Tyr Leu Gly Val Leu Ala
2835 2840 2845

ggg agt tcg gga gcg aat gat gcg cca gca ttg att tct cgc ctg ttg 10620
Gly Ser Ser Gly Ala Asn Asp Ala Pro Ala Leu Ile Ser Arg Leu Leu
2850 2855 2860 2865

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ctg gac tac agg gaa agt cct tca ctc agt ctg ctc gag aac gtg cac    10668
Leu Asp Tyr Arg Glu Ser Pro Ser Leu Ser Leu Leu Glu Asn Val His
            2870                2875                2880

cag gtg gct tat gag tcg gac ggg acg tct tgt gcc ttg ccg gca ctg    10716
Gln Val Ala Tyr Glu Ser Asp Gly Thr Ser Cys Ala Leu Pro Ala Leu
        2885                2890                2895

gca ttg ggg tgg caa acc ttt acc ccg ccg aca ttg tcg gca tgg cag    10764
Ala Leu Gly Trp Gln Thr Phe Thr Pro Pro Thr Leu Ser Ala Trp Gln
    2900                2905                2910

acg cgt gac gat atg ggc aag ttg agt ttg ctt caa ccc tat cag ctt    10812
Thr Arg Asp Asp Met Gly Lys Leu Ser Leu Leu Gln Pro Tyr Gln Leu
2915                2920                2925

gta gac ctt aac ggc gaa ggt gtg gtg ggt atc ctg tat cag gac agc    10860
Val Asp Leu Asn Gly Glu Gly Val Val Gly Ile Leu Tyr Gln Asp Ser
2930                2935                2940                2945

ggt gcc tgg tgg tac cgt gaa ccg gta cgc cag tcg ggg gat gat ccg    10908
Gly Ala Trp Trp Tyr Arg Glu Pro Val Arg Gln Ser Gly Asp Asp Pro
            2950                2955                2960

gat gct gtg acc tgg ggg gcg gct gcg gcc ctg ccg aca atg ccc gct    10956
Asp Ala Val Thr Trp Gly Ala Ala Ala Ala Leu Pro Thr Met Pro Ala
        2965                2970                2975

ttg cat aac agc ggc atc ctg gcg gat ctt aat ggg gat ggt cgg ctg    11004
Leu His Asn Ser Gly Ile Leu Ala Asp Leu Asn Gly Asp Gly Arg Leu
    2980                2985                2990

gag tgg gtc gtt acc gcc ccc ggt gtg gcg ggg atg tat gat cgc acc    11052
Glu Trp Val Val Thr Ala Pro Gly Val Ala Gly Met Tyr Asp Arg Thr
2995                3000                3005

ccc ggc cgc gac tgg ttg cat ttc acc ccc ctg tca gcc ttg ccc gta    11100
Pro Gly Arg Asp Trp Leu His Phe Thr Pro Leu Ser Ala Leu Pro Val
3010                3015                3020                3025

gaa tat gcg cat cca aaa gca gtg ctc gcc gat atc ctg ggg gct ggg    11148
Glu Tyr Ala His Pro Lys Ala Val Leu Ala Asp Ile Leu Gly Ala Gly
            3030                3035                3040

tta acg gac atg gtg ctt atc ggg ccg cgc agt gtt cgc ctc tat tcc    11196
Leu Thr Asp Met Val Leu Ile Gly Pro Arg Ser Val Arg Leu Tyr Ser
        3045                3050                3055

ggc aaa aac gat ggt tgg aat aaa ggg gag acc gtg cag caa acg gaa    11244
Gly Lys Asn Asp Gly Trp Asn Lys Gly Glu Thr Val Gln Gln Thr Glu
    3060                3065                3070

aga ctc act ctg ccg gtc ccg ggg gtt gac cca cgt acc ctc gtg gcg    11292
Arg Leu Thr Leu Pro Val Pro Gly Val Asp Pro Arg Thr Leu Val Ala
3075                3080                3085

ttc agt gat atg gct ggc agt gga cag cag cat ttg acg gag gtg cgt    11340
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Phe Ser Asp Met Ala Gly Ser Gly Gln Gln His Leu Thr Glu Val Arg
3090 3095 3100 3105

gct aat gga gta cgt tac tgg cca aac ctg ggg cac ggt cgt ttc ggt 11388
Ala Asn Gly Val Arg Tyr Trp Pro Asn Leu Gly His Gly Arg Phe Gly
3110 3115 3120

cag ccg gtg aat att ccc ggt ttt agc cag tca gtg act acg ttt aac 11436
Gln Pro Val Asn Ile Pro Gly Phe Ser Gln Ser Val Thr Thr Phe Asn
3125 3130 3135

cct gac cag ata ttg ctg gcc gat acc gac ggt tcc ggt acc acg gac 11484
Pro Asp Gln Ile Leu Leu Ala Asp Thr Asp Gly Ser Gly Thr Thr Asp
3140 3145 3150

ctg att tat gcg atg agt gac cgg tta gtc att tat ttc aac cag agt 11532
Leu Ile Tyr Ala Met Ser Asp Arg Leu Val Ile Tyr Phe Asn Gln Ser
3155 3160 3165

ggt aat tat ttc gcc gag ccg cat acg ctg ctc ttg ccg aaa ggt gtg 11580
Gly Asn Tyr Phe Ala Glu Pro His Thr Leu Leu Leu Pro Lys Gly Val
3170 3175 3180 3185

cgc tat gat cgc acc tgc agt ctg caa gtg gcg gat atc cag ggg ctg 11628
Arg Tyr Asp Arg Thr Cys Ser Leu Gln Val Ala Asp Ile Gln Gly Leu
3190 3195 3200

ggg gtg cct agc ctg tta ctg acg gtc ccc cat gtc gcg cct cat cac 11676
Gly Val Pro Ser Leu Leu Leu Thr Val Pro His Val Ala Pro His His
3205 3210 3215

tgg gtg tgc cat tta tcg gca gac aaa ccc tgg ttg ttg aat ggc atg 11724
Trp Val Cys His Leu Ser Ala Asp Lys Pro Trp Leu Leu Asn Gly Met
3220 3225 3230

aac aac aat atg ggg gcc cgg cat gca ctg cac tat cgc agt tcg gtg 11772
Asn Asn Asn Met Gly Ala Arg His Ala Leu His Tyr Arg Ser Ser Val
3235 3240 3245

cag ttc tgg ctg gat gag aaa gcc gag gca ctg gcg gca ggc agt tcc 11820
Gln Phe Trp Leu Asp Glu Lys Ala Glu Ala Leu Ala Ala Gly Ser Ser
3250 3255 3260 3265

cct gcc tgc tac ctg cca ttt aca ttg cat acc ctg tgg cgt tcg gtg 11868
Pro Ala Cys Tyr Leu Pro Phe Thr Leu His Thr Leu Trp Arg Ser Val
3270 3275 3280

gtg cag gat gag atc acc ggt aac cgt ctg gtc agc gac gtg ctt tat 11916
Val Gln Asp Glu Ile Thr Gly Asn Arg Leu Val Ser Asp Val Leu Tyr
3285 3290 3295

cgc cac ggc gtc tgg gac ggg cag gaa cgc gag ttt cgg ggg ttt ggt 11964
Arg His Gly Val Trp Asp Gly Gln Glu Arg Glu Phe Arg Gly Phe Gly
3300 3305 3310

ttt gtt gag atc agg gat acc gat acc ttg gca agc cag ggt acg gcg 12012
Phe Val Glu Ile Arg Asp Thr Asp Thr Leu Ala Ser Gln Gly Thr Ala
3315 3320 3325

acg gaa ctg agt atg cct tct gtg agc cgg aac tgg tat gcc acc ggg 12060
Thr Glu Leu Ser Met Pro Ser Val Ser Arg Asn Trp Tyr Ala Thr Gly
3330 3335 3340 3345

gta ccg gca gta gac gag cgt ctg ccg gag acg tat tgg caa aac gat 12108
Val Pro Ala Val Asp Glu Arg Leu Pro Glu Thr Tyr Trp Gln Asn Asp
3350 3355 3360

gcc gcc gct ttt gcc gat ttc gcg acc cgt ttc act gtc ggt tca gga 12156
Ala Ala Ala Phe Ala Asp Phe Ala Thr Arg Phe Thr Val Gly Ser Gly
3365 3370 3375

gag gat gag cag aca tat act ccg gac gac agc aag aca ttc tgg ttg 12204
Glu Asp Glu Gln Thr Tyr Thr Pro Asp Asp Ser Lys Thr Phe Trp Leu
3380 3385 3390

cag cga gcc ctg aaa ggc atc ctg ctg cgc agt gag tta tac ggt gcc 12252
Gln Arg Ala Leu Lys Gly Ile Leu Leu Arg Ser Glu Leu Tyr Gly Ala
3395 3400 3405

gat ggc agc agc cag gcc gat atc cct tac agc gtc act gag tct cgc 12300
Asp Gly Ser Ser Gln Ala Asp Ile Pro Tyr Ser Val Thr Glu Ser Arg
3410 3415 3420 3425

ccg cag gta cgg cta gtt gaa gcg aat gga gac tac ccg gtg gtg tgg 12348
Pro Gln Val Arg Leu Val Glu Ala Asn Gly Asp Tyr Pro Val Val Trp
3430 3435 3440

ccg atg ggc gcg gaa agc cgt acg tca gtt tat gaa cgg tac cac aat 12396
Pro Met Gly Ala Glu Ser Arg Thr Ser Val Tyr Glu Arg Tyr His Asn
3445 3450 3455

gat cct caa tgc caa cag cag gcg gta ctc ctc agt gat gaa tac ggt 12444
Asp Pro Gln Cys Gln Gln Gln Ala Val Leu Leu Ser Asp Glu Tyr Gly
3460 3465 3470

ttc cca ctg cgt cag gtc agt gtc aat tat cca cga cgc cct ccg tcg 12492
Phe Pro Leu Arg Gln Val Ser Val Asn Tyr Pro Arg Arg Pro Pro Ser
3475 3480 3485

gcg gac aat cca tat ccg gcg tcc tta ccg gcg acg ctg ttc gcc aac 12540
Ala Asp Asn Pro Tyr Pro Ala Ser Leu Pro Ala Thr Leu Phe Ala Asn
3490 3495 3500 3505

agt tat gac gag cag cag cag ata tta cgc ctg ggg ttg caa cag agc 12588
Ser Tyr Asp Glu Gln Gln Gln Ile Leu Arg Leu Gly Leu Gln Gln Ser
3510 3515 3520

agt gca cat cac ctt gtt tca ctg tct gag ggg cat tgg ttg ttg ggg 12636
Ser Ala His His Leu Val Ser Leu Ser Glu Gly His Trp Leu Leu Gly
3525 3530 3535

ttg gcg gag gcg tcg cgg gac gat gta ttc acg tac tct gcg gac aac 12684
Leu Ala Glu Ala Ser Arg Asp Asp Val Phe Thr Tyr Ser Ala Asp Asn
3540 3545 3550

gtg ccg gaa ggg ggt ctg acg ctg gaa cac ctg ttg gcg ccc gaa agc 12732

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Val Pro Glu Gly Gly Leu Thr Leu Glu His Leu Leu Ala Pro Glu Ser
    3555                3560                3565

ctg gtc tcg gat agt cag gtc ggt acg ctg gcg ggt cag cag caa gtc     12780
Leu Val Ser Asp Ser Gln Val Gly Thr Leu Ala Gly Gln Gln Gln Val
3570                3575                3580                3585

tgg tat ctg gat tca caa gac gtt gcc acc gtc gct gct ccg cca ctc     12828
Trp Tyr Leu Asp Ser Gln Asp Val Ala Thr Val Ala Ala Pro Pro Leu
                3590                3595                3600

ccc ccc aag gta gct ttt atc gaa acg gcc gtg ctg gat gag ggt atg     12876
Pro Pro Lys Val Ala Phe Ile Glu Thr Ala Val Leu Asp Glu Gly Met
            3605                3610                3615

gtc agt tca ctg gct gcc tac att gtg gat gaa cat ctc gag caa gcc     12924
Val Ser Ser Leu Ala Ala Tyr Ile Val Asp Glu His Leu Glu Gln Ala
        3620                3625                3630

ggt tac cgg caa tcc gga tac ctt ttc cct cga ggc agg gaa gca gaa     12972
Gly Tyr Arg Gln Ser Gly Tyr Leu Phe Pro Arg Gly Arg Glu Ala Glu
    3635                3640                3645

cag gca ttg tgg acc cag tgt cag gga tat gtt acc tat gcc ggc gca     13020
Gln Ala Leu Trp Thr Gln Cys Gln Gly Tyr Val Thr Tyr Ala Gly Ala
3650                3655                3660                3665

gag cat ttc tgg cta ccg cta tcc ttt cgg gac agt atg ttg acc ggc     13068
Glu His Phe Trp Leu Pro Leu Ser Phe Arg Asp Ser Met Leu Thr Gly
                3670                3675                3680

cca gtt acc gtg acg cgt gac gcg tac gac tgc gtc atc acg cag tgg     13116
Pro Val Thr Val Thr Arg Asp Ala Tyr Asp Cys Val Ile Thr Gln Trp
            3685                3690                3695

cag gat gcc gca ggg att gtc acc aca gcc gac tat gac tgg cgc ttc     13164
Gln Asp Ala Ala Gly Ile Val Thr Thr Ala Asp Tyr Asp Trp Arg Phe
        3700                3705                3710

ctg acg ccc gtc cgg gtg acg gac ccc aat gat aat ctg cag tcc gtc     13212
Leu Thr Pro Val Arg Val Thr Asp Pro Asn Asp Asn Leu Gln Ser Val
    3715                3720                3725

act ctg gat gct ctg ggc cgg gtg acc acc ctg cga ttc tgg ggc acg     13260
Thr Leu Asp Ala Leu Gly Arg Val Thr Thr Leu Arg Phe Trp Gly Thr
3730                3735                3740                3745

gag aat ggt att gcc acc ggt tac agt gat gcc acg ttg tcc gtt ccg     13308
Glu Asn Gly Ile Ala Thr Gly Tyr Ser Asp Ala Thr Leu Ser Val Pro
                3750                3755                3760

gac ggc gca gca gcc gct ctg gcg ttg acg gcg ccc cta cca gta gca     13356
Asp Gly Ala Ala Ala Ala Leu Ala Leu Thr Ala Pro Leu Pro Val Ala
            3765                3770                3775

cag tgt ctg gtg tat gtc acg gac agt tgg gga gat gac gac aat gag     13404
Gln Cys Leu Val Tyr Val Thr Asp Ser Trp Gly Asp Asp Asp Asn Glu
        3780                3785                3790
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aaa atg ccc ccg cac gtg gtc gtg ctg gct acc gat cgc tat gac agt   13452
Lys Met Pro Pro His Val Val Val Leu Ala Thr Asp Arg Tyr Asp Ser
   3795                3800                3805

gat acc gga cag cag gtc cgc caa cag gtg aca ttc agt gac ggt ttt   13500
Asp Thr Gly Gln Gln Val Arg Gln Gln Val Thr Phe Ser Asp Gly Phe
3810                3815                3820                3825

ggg cgt gag ttg caa tcg gca acc cgg cag gcc gag ggc aac gcc tgg   13548
Gly Arg Glu Leu Gln Ser Ala Thr Arg Gln Ala Glu Gly Asn Ala Trp
                3830                3835                3840

caa cga gga cgc gac ggc aaa ctg gtg acg gcc agt gac gga ttg ccg   13596
Gln Arg Gly Arg Asp Gly Lys Leu Val Thr Ala Ser Asp Gly Leu Pro
            3845                3850                3855

gtc act gta gca acg aat ttc cgc tgg gcg gtc acc ggg agg gcg gag   13644
Val Thr Val Ala Thr Asn Phe Arg Trp Ala Val Thr Gly Arg Ala Glu
        3860                3865                3870

tat gac aat aaa ggt ctg cct gtt cgg gtt tat cag ccg tat ttt ctg   13692
Tyr Asp Asn Lys Gly Leu Pro Val Arg Val Tyr Gln Pro Tyr Phe Leu
    3875                3880                3885

gac agt tgg caa tat gtc agt gat gac agt gcc cgc cag gac ctg tat   13740
Asp Ser Trp Gln Tyr Val Ser Asp Asp Ser Ala Arg Gln Asp Leu Tyr
3890                3895                3900                3905

gcc gac acg cac ttt tac gat ccg acg gca cgg gaa tgg cag gtt att   13788
Ala Asp Thr His Phe Tyr Asp Pro Thr Ala Arg Glu Trp Gln Val Ile
                3910                3915                3920

acg gca aaa ggt gaa cgg cga cag gtg ctg tat acc ccg tgg ttt gtg   13836
Thr Ala Lys Gly Glu Arg Arg Gln Val Leu Tyr Thr Pro Trp Phe Val
            3925                3930                3935

gtc agt gaa gac gag aat gat acc gtt ggg cta aac gac gca tcc tga   13884
Val Ser Glu Asp Glu Asn Asp Thr Val Gly Leu Asn Asp Ala Ser
        3940                3945                3950

ctgggaagga gggggggacg gtg atg agt ccg tcg ccc ctg aca ggc gct gcc 13937
                          Met Ser Pro Ser Pro Leu Thr Gly Ala Ala
                              3955                3960

ctg atg gag aca aag atg aaa ata cac tat cag gtt gcg gcg gtt gtg   13985
Leu Met Glu Thr Lys Met Lys Ile His Tyr Gln Val Ala Ala Val Val
    3965                3970                3975

ctg aca ggt gtt atg gtt tgg ggg ctt tcc cat tgg cgt tac acc gtc   14033
Leu Thr Gly Val Met Val Trp Gly Leu Ser His Trp Arg Tyr Thr Val
3980                3985                3990                3995

ggt tac cac gcg gca gat act caa tgg caa caa cgc cag gcc gaa cag   14081
Gly Tyr His Ala Ala Asp Thr Gln Trp Gln Gln Arg Gln Ala Glu Gln
                4000                4005                4010

gaa agg gcc gat gcg ttg gcc ctc ctg gca gca gaa acc cgg gaa aga   14129
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Glu Arg Ala Asp Ala Leu Ala Leu Leu Ala Ala Glu Thr Arg Glu Arg
4015 4020 4025

aag tgg gag cag caa cga cag act gac atg aac aag gtg gct ata cat 14177
Lys Trp Glu Gln Gln Arg Gln Thr Asp Met Asn Lys Val Ala Ile His
4030 4035 4040

gct gaa gaa gaa ctg gct gct gcg cgt gac gct gcc gct gat gct cag 14225
Ala Glu Glu Glu Leu Ala Ala Ala Arg Asp Ala Ala Ala Asp Ala Gln
4045 4050 4055

cgc act ggt cag cgc ctg cag cac acc gtt acc acc ctc cag cgg caa 14273
Arg Thr Gly Gln Arg Leu Gln His Thr Val Thr Thr Leu Gln Arg Gln
4060 4065 4070 4075

ctt gcc agt cgt gaa acc cgc cgc ctt tcc gca gct acc gct atc ggt 14321
Leu Ala Ser Arg Glu Thr Arg Arg Leu Ser Ala Ala Thr Ala Ile Gly
4080 4085 4090

aca gac gac ctc gga ggc caa ccc ggc gtt ttg ttt gcc gaa ctg ttc 14369
Thr Asp Asp Leu Gly Gly Gln Pro Gly Val Leu Phe Ala Glu Leu Phe
4095 4100 4105

cgc cgc gct gac cag aga gcg gga gag ctg gca gcg tat gct gac agg 14417
Arg Arg Ala Asp Gln Arg Ala Gly Glu Leu Ala Ala Tyr Ala Asp Arg
4110 4115 4120

acc aga gtg aaa tgg cag gcc tgc ggg cgc gcc tat cag gcg gct acg 14465
Thr Arg Val Lys Trp Gln Ala Cys Gly Arg Ala Tyr Gln Ala Ala Thr
4125 4130 4135

cac gaa gca gaa aaa taa ggcgatttag ccgttaagga aaagtgacgg 14513
His Glu Ala Glu Lys
4140 4145

tgttttcgcg attaatatta acaggagatc ac atg agc aca tcc ttg ttc agt 14566
Met Ser Thr Ser Leu Phe Ser
4150

agc acc ccg tcg gtc gcg gtg ctc gac aac cgc ggc ctg ttg gtg cgg 14614
Ser Thr Pro Ser Val Ala Val Leu Asp Asn Arg Gly Leu Leu Val Arg
4155 4160 4165

gag ctg cag tac tac cgc cat ccg gat aca ccg gag gag acg gac gag 14662
Glu Leu Gln Tyr Tyr Arg His Pro Asp Thr Pro Glu Glu Thr Asp Glu
4170 4175 4180

cgt atc acc tgc cat cag cac gat gag cgc ggc agc ttg tca caa agc 14710
Arg Ile Thr Cys His Gln His Asp Glu Arg Gly Ser Leu Ser Gln Ser
4185 4190 4195 4200

gcc gac ccg cgg tta cac gcg gcc ggt ctg aca aat ttc acg tac ctg 14758
Ala Asp Pro Arg Leu His Ala Ala Gly Leu Thr Asn Phe Thr Tyr Leu
4205 4210 4215

aat agc ctg acc ggg aca gta ctg cag agc gtc agc gcc gat gcc ggt 14806
Asn Ser Leu Thr Gly Thr Val Leu Gln Ser Val Ser Ala Asp Ala Gly
4220 4225 4230

acg tcg ctg gaa ctg agc gat gcc gcc ggg cgg gcg ttt ctg gcc gtc 14854
Thr Ser Leu Glu Leu Ser Asp Ala Ala Gly Arg Ala Phe Leu Ala Val
4235 4240 4245

acc ggg gct ggg acg gaa gac gcg gtc acc cgc acc tgg caa tat gaa 14902
Thr Gly Ala Gly Thr Glu Asp Ala Val Thr Arg Thr Trp Gln Tyr Glu
4250 4255 4260

gac gat acc ctg ccg ggc cgc ccg ctg agc atc acc gag cag gtt acc 14950
Asp Asp Thr Leu Pro Gly Arg Pro Leu Ser Ile Thr Glu Gln Val Thr
4265 4270 4275 4280

ggt gaa gcc gcc caa att acg gaa cgc ttc gtg tac gct ggc aat acg 14998
Gly Glu Ala Ala Gln Ile Thr Glu Arg Phe Val Tyr Ala Gly Asn Thr
4285 4290 4295

gat gcc gag aag att ctc aat ctg gct ggc cag tgt gtc agt cat tac 15046
Asp Ala Glu Lys Ile Leu Asn Leu Ala Gly Gln Cys Val Ser His Tyr
4300 4305 4310

gat acc gcc gga ctg gtg cag acg gac agc atc gcc ctg agc ggc gtg 15094
Asp Thr Ala Gly Leu Val Gln Thr Asp Ser Ile Ala Leu Ser Gly Val
4315 4320 4325

ccg ctc gcc gtc acg cgg cag ttg ctg ccc gac gcg gcg ggg gcc aac 15142
Pro Leu Ala Val Thr Arg Gln Leu Leu Pro Asp Ala Ala Gly Ala Asn
4330 4335 4340

tgg atg ggt gag gat gcc tcg gcc tgg aat gac ctg ctg gat ggg gag 15190
Trp Met Gly Glu Asp Ala Ser Ala Trp Asn Asp Leu Leu Asp Gly Glu
4345 4350 4355 4360

acg ttc ttc acc cag acc cac gct gat gcg acc ggc gcc gtc ctg agc 15238
Thr Phe Phe Thr Gln Thr His Ala Asp Ala Thr Gly Ala Val Leu Ser
4365 4370 4375

atc acc gat gca aaa ggt aat ctg cag cgt gtg gca tat gat gtg gct 15286
Ile Thr Asp Ala Lys Gly Asn Leu Gln Arg Val Ala Tyr Asp Val Ala
4380 4385 4390

ggg ctg cta tcg ggc agt tgg ttg acg ctg aag gac ggc acg gag cag 15334
Gly Leu Leu Ser Gly Ser Trp Leu Thr Leu Lys Asp Gly Thr Glu Gln
4395 4400 4405

gtc atc gtg gcc tcc ctg acg tac tcg gcc gcc ggg aaa aag ttg cgt 15382
Val Ile Val Ala Ser Leu Thr Tyr Ser Ala Ala Gly Lys Lys Leu Arg
4410 4415 4420

gaa gaa cac ggc aac ggc gtg gta acc tcg tat att tac gag ccg gaa 15430
Glu Glu His Gly Asn Gly Val Val Thr Ser Tyr Ile Tyr Glu Pro Glu
4425 4430 4435 4440

aca cag cgc ctg acg ggg att aaa acg gaa cgt ccg tct ggg cac gtt 15478
Thr Gln Arg Leu Thr Gly Ile Lys Thr Glu Arg Pro Ser Gly His Val
4445 4450 4455

gcc gga gca aaa gtg ctg cag gac ctg cgc tat acg tat gac ccg gta 15526

```
Ala Gly Ala Lys Val Leu Gln Asp Leu Arg Tyr Thr Tyr Asp Pro Val
            4460                4465                4470

ggc aac gta ctc agc gtc aat aac gat gcg gaa gag acc cgc ttc tgg   15574
Gly Asn Val Leu Ser Val Asn Asn Asp Ala Glu Glu Thr Arg Phe Trp
        4475                4480                4485

cgt aac cag aaa gtg gta ccg gag aat acg tac atc tac gac agc ctg   15622
Arg Asn Gln Lys Val Val Pro Glu Asn Thr Tyr Ile Tyr Asp Ser Leu
    4490                4495                4500

tac cag ctg gtc agc gcc aca ggg cgt gag atg gcc aat gcc ggc cag   15670
Tyr Gln Leu Val Ser Ala Thr Gly Arg Glu Met Ala Asn Ala Gly Gln
4505                4510                4515                4520

cag ggc aac gac tta cca tcc gct aca gcc ccc ctt cct aca gac agc   15718
Gln Gly Asn Asp Leu Pro Ser Ala Thr Ala Pro Leu Pro Thr Asp Ser
                4525                4530                4535

tct gcc tac acc aat tac acg cgc acc tac cgt tat gac cgt ggt ggc   15766
Ser Ala Tyr Thr Asn Tyr Thr Arg Thr Tyr Arg Tyr Asp Arg Gly Gly
            4540                4545                4550

aac ctg acg cag atg cgc cac agt gcc cct gcc acg aac aat aat tat   15814
Asn Leu Thr Gln Met Arg His Ser Ala Pro Ala Thr Asn Asn Asn Tyr
        4555                4560                4565

acg aca gac atc acg gtt agt gac cgc agc aat agg gcg gta ctg agc   15862
Thr Thr Asp Ile Thr Val Ser Asp Arg Ser Asn Arg Ala Val Leu Ser
    4570                4575                4580

acg ttg gcg gaa gtg ccg tca gat gtt gat atg ctg ttc agt gca gga   15910
Thr Leu Ala Glu Val Pro Ser Asp Val Asp Met Leu Phe Ser Ala Gly
4585                4590                4595                4600

ggt cac cag aag cac ctg cag ccg ggg caa gca ctg gtg tgg acg cca   15958
Gly His Gln Lys His Leu Gln Pro Gly Gln Ala Leu Val Trp Thr Pro
                4605                4610                4615

cgt gga gaa ctg caa aag gtg aca ccg gtg gtg cgt gat ggg ggg gcg   16006
Arg Gly Glu Leu Gln Lys Val Thr Pro Val Val Arg Asp Gly Gly Ala
            4620                4625                4630

gac gac agc gaa agc tat cgg tat gat gcg ggc agt cag cgt att atc   16054
Asp Asp Ser Glu Ser Tyr Arg Tyr Asp Ala Gly Ser Gln Arg Ile Ile
        4635                4640                4645

aaa acc ggc acg cgg caa act ggc aac aac gtt cag aca cag cgg gta   16102
Lys Thr Gly Thr Arg Gln Thr Gly Asn Asn Val Gln Thr Gln Arg Val
    4650                4655                4660

gtg tac ctg ccg ggg ctg gag tta cgt atc atg gca aat ggc gtg acg   16150
Val Tyr Leu Pro Gly Leu Glu Leu Arg Ile Met Ala Asn Gly Val Thr
4665                4670                4675                4680

gaa aaa gaa agc ctg cag gtt att acg gtg ggc gag gct ggg cgg gca   16198
Glu Lys Glu Ser Leu Gln Val Ile Thr Val Gly Glu Ala Gly Arg Ala
                4685                4690                4695
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caa gtg cgc gta ttg cac tgg gag atc ggc aag ccg gat gac ctc gat 16246
Gln Val Arg Val Leu His Trp Glu Ile Gly Lys Pro Asp Asp Leu Asp
4700 4705 4710

gag gac tcg gtg cgt tac agt tac gat aac ctg gtg ggc agc agc cag 16294
Glu Asp Ser Val Arg Tyr Ser Tyr Asp Asn Leu Val Gly Ser Ser Gln
4715 4720 4725

ctg gag ctg gac aga gag ggt tac ctt atc agt gag gag gag ttc tac 16342
Leu Glu Leu Asp Arg Glu Gly Tyr Leu Ile Ser Glu Glu Glu Phe Tyr
4730 4735 4740

ccg tat ggc gga acg gct gtt ctg acg gcg cga agt gag gtt gag gct 16390
Pro Tyr Gly Gly Thr Ala Val Leu Thr Ala Arg Ser Glu Val Glu Ala
4745 4750 4755 4760

gac tac aaa act atc cga tac tca ggc aag gag cgt gac gcg acg ggg 16438
Asp Tyr Lys Thr Ile Arg Tyr Ser Gly Lys Glu Arg Asp Ala Thr Gly
4765 4770 4775

ctg gat tat tac ggt tat cgg tat tac cag cca tgg gca ggg cgc tgg 16486
Leu Asp Tyr Tyr Gly Tyr Arg Tyr Tyr Gln Pro Trp Ala Gly Arg Trp
4780 4785 4790

ctc tcc acg gac ccg gca ggc acg gtg gac ggg ctg aac ctg ttc cgc 16534
Leu Ser Thr Asp Pro Ala Gly Thr Val Asp Gly Leu Asn Leu Phe Arg
4795 4800 4805

atg gtg cgg aat aat ccc gtc acg ctg ttt gac agc aac ggg cgg atc 16582
Met Val Arg Asn Asn Pro Val Thr Leu Phe Asp Ser Asn Gly Arg Ile
4810 4815 4820

agt act ggt cag gag gcc aga cga tta gtg ggg gaa gca ttt gtt cat 16630
Ser Thr Gly Gln Glu Ala Arg Arg Leu Val Gly Glu Ala Phe Val His
4825 4830 4835 4840

ccg tta cac atg cct gtt ttt gaa aga att tct gta gag aga aag att 16678
Pro Leu His Met Pro Val Phe Glu Arg Ile Ser Val Glu Arg Lys Ile
4845 4850 4855

tca atg agc gta agg gaa gct ggc att tat act att tca gcg ctg ggt 16726
Ser Met Ser Val Arg Glu Ala Gly Ile Tyr Thr Ile Ser Ala Leu Gly
4860 4865 4870

gaa ggt gca gca gca aaa ggc cat aat att cta gag aaa acc att aaa 16774
Glu Gly Ala Ala Ala Lys Gly His Asn Ile Leu Glu Lys Thr Ile Lys
4875 4880 4885

ccc ggt tcc ctg aag gct atc tat ggt gat aaa gct gag tca att ctt 16822
Pro Gly Ser Leu Lys Ala Ile Tyr Gly Asp Lys Ala Glu Ser Ile Leu
4890 4895 4900

gga ctg gca aaa cgt agc ggt ctc gtt ggc cga gta gga cag tgg gat 16870
Gly Leu Ala Lys Arg Ser Gly Leu Val Gly Arg Val Gly Gln Trp Asp
4905 4910 4915 4920

gca tca ggt gta cgt gga att tat gcg cac aac aga ccg ggt ggt gag 16918

Ala Ser Gly Val Arg Gly Ile Tyr Ala His Asn Arg Pro Gly Gly Glu
4925 4930 4935

gat ttg gtt tat cct gtc agc ctg cag aat act tct gcc aat gaa att 16966
Asp Leu Val Tyr Pro Val Ser Leu Gln Asn Thr Ser Ala Asn Glu Ile
4940 4945 4950

gtt aat gca tgg ata aaa ttt aaa atc atc acg ccc tac acc ggg gat 17014
Val Asn Ala Trp Ile Lys Phe Lys Ile Ile Thr Pro Tyr Thr Gly Asp
4955 4960 4965

tat gac atg cac gat att att aaa ttc tct gat ggg aaa ggg cat gtg 17062
Tyr Asp Met His Asp Ile Ile Lys Phe Ser Asp Gly Lys Gly His Val
4970 4975 4980

cct aca gcg gaa agt agt gag gaa aga gga gta aaa gat cta att aat 17110
Pro Thr Ala Glu Ser Ser Glu Glu Arg Gly Val Lys Asp Leu Ile Asn
4985 4990 4995 5000

aaa ggt gtt gcg gag gtc gat cct tcc aga ccc ttt gag tat aca gcg 17158
Lys Gly Val Ala Glu Val Asp Pro Ser Arg Pro Phe Glu Tyr Thr Ala
5005 5010 5015

atg aat gtt att cgc cat gga cca cag gtg aac ttt gtt ccc tat atg 17206
Met Asn Val Ile Arg His Gly Pro Gln Val Asn Phe Val Pro Tyr Met
5020 5025 5030

tgg gaa cat gag cac gat aaa gtc gtt aat gat aat ggt tat ctg ggg 17254
Trp Glu His Glu His Asp Lys Val Val Asn Asp Asn Gly Tyr Leu Gly
5035 5040 5045

gtg gta gct agc ccg ggg ccg ttc ccg gta gcg atg gta cat cag ggg 17302
Val Val Ala Ser Pro Gly Pro Phe Pro Val Ala Met Val His Gln Gly
5050 5055 5060

gaa tgg act gtt ttt gac aac agt gaa gaa ctg ttt aat ttc tat aaa 17350
Glu Trp Thr Val Phe Asp Asn Ser Glu Glu Leu Phe Asn Phe Tyr Lys
5065 5070 5075 5080

tct aca aat aca cct ctt cct gaa cac tgg tcc caa gat ttt atg gac 17398
Ser Thr Asn Thr Pro Leu Pro Glu His Trp Ser Gln Asp Phe Met Asp
5085 5090 5095

aga ggg aaa gga ata gtc gca act cct cgg cat gct gaa ctt ctt gat 17446
Arg Gly Lys Gly Ile Val Ala Thr Pro Arg His Ala Glu Leu Leu Asp
5100 5105 5110

aaa cga cga gtc atg tac taa tcgtaacgat ttcctgcctt acccaaagta 17497
Lys Arg Arg Val Met Tyr
5115

tacagcccgg tgagacattt tctctgtctc atttgggttg tttttgtctc atctgcatgt 17557

tatgtcttcc ctcatctaaa gtctaacgag acatttttag caaaatggca ctttacggtt 17617

atgttcgcgt ttcaaccgac ggtccggatt ttactctgta aatacagaca cttcgcgcag 17677

cctgctgcga aattatccgt gcgaaaaaag ccagcggcag cagccgggat ggacgaaatg 17737

aactgcagct tctgctggct tttttgcggc caggcaacat gctgatggtt acgtgagttg 17797

atcggctgcc accaaaaagt ccggagcgtg cggcccagat cgccgcaata atactgctgt 17857

atggtatttc catcaccact gtatatcgca cactctgggc cttccagaaa ccccataccg 17917

cacaccggtg tgatcgctgg aagccccggg cattaccgcc gtctgtactc gaacactatt 17977

gtggacttga tggttaggag attgaatcga ccatttttga gatccctaac catagatcgt 18037

agagttgcac actcccagat ggcgtggctt agcgagcgat tatgcttaaa aattcatgtt 18097

ttgctgtgtt tttaatccaa aacctgcttt tcaggcgcac ttatccagct acggggtctg 18157

aagccatcgt ttttttgccg tacgatgtag cctgtcagag agcatttttg tggcgtgctc 18217

gcccgctacg gtaccggcgg caaaacgcag ccggcctttg cagaggatgc actggtacgg 18277

atcggtgccc aggaagcctt tcatcagcac cgcgaacccg ggccgtttcg gtttctcccg 18337

taccgtcatc tccagcgcgt cgtaaacctt cggcagcagc gtgcccgttt gcggttggcc 18397

agaaaaccat agtaacgcac cattttaaaa tgccgtgcag ggatatggct gacgtaacgc 18457

tgcagcatct cctcctggct gattttctgg cgtttgtgct gctgcgtacg gtgatcgtaa 18517

tactgatgca ccacggcccc gccgcggtag tggcgtagct gagaagccgc caccggcggg 18577

cgcttcaggt accgggccag gtatttcacg ctgcgccagg cgccgcgggt ctttttggca 18637

aaattcactt tccaggggcg gcggtattgc gcatgcaggg tcttcgttgc ggatatggcc 18697

gagacccggc agggcgccag gattgatgcg cagcaggtga acgacggcat tgcgccagat 18757

ggcttccacc tctttctttt taaagaacag ctgccgccag acgtggtgtt tgacgtcaag 18817

accgccgcgg gtaacggaga cgtggatatg cggatgttga ttgagctgcc ggccttaggt 18877

gtggagcgcg caaaaaatgc cggcctcgat gccctgccgg cgtgcccagc ggagcatggc 18937

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 144 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: PROTEIN (ORF 1)

(ix) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

```
Met Lys Ile Ser Ser Arg Gly Ile Ala Leu Ile Lys Glu Phe Glu Gly
 1               5                  10                  15

Leu Arg Leu His Ala Tyr Arg Cys Ala Ala Asp Val Trp Thr Val Gly
            20                  25                  30

Tyr Gly His Thr Ala Gly Val Thr Lys Gly Asp Ile Ile Thr Val Asp
        35                  40                  45

Glu Ala Gln Thr Met Leu Thr Asn Asp Ile Thr Val Phe Glu Arg Ala
    50                  55                  60

Val Ser Gln Ala Val Ala Val Pro Leu Asn Gln Ser Gln Tyr Asp Ala
65                  70                  75                  80

Leu Val Ser Leu Val Phe Asn Ile Gly Gln Gly Asn Phe Lys Arg Ser
                85                  90                  95

Thr Leu Leu Lys Lys Leu Asn Lys Gln Asp Tyr Val Gly Ala Gly Asn
            100                 105                 110

Glu Phe Leu Arg Trp Thr Arg Ala Asn Gly Lys Val Leu Pro Gly Leu
        115                 120                 125

Ile Arg Arg Arg Glu Ala Glu Arg Val Leu Phe Glu Lys Leu Gly
    130                 135                 140

Ala
```

(2) INFORMATION FOR SEQ ID NO: 3:

(i). SEQUENCE CHARACTERISTICS:
(A) LENGTH: 191 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: PROTEIN (ORF 2)

(ix) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Met Ser Pro Ser Pro Leu Thr Gly Ala Ala Leu Met Glu Thr Lys Met
1 5 10 15

Lys Ile His Tyr Gln Val Ala Ala Val Val Leu Thr Gly Val Met Val
20 25 30

Trp Gly Leu Ser His Trp Arg Tyr Thr Val Gly Tyr His Ala Ala Asp
35 40 45

Thr Gln Trp Gln Gln Arg Gln Ala Glu Gln Glu Arg Ala Asp Ala Leu
50 55 60

Ala Leu Leu Ala Ala Glu Thr Arg Glu Arg Lys Trp Glu Gln Gln Arg
65 70 75 80

Gln Thr Asp Met Asn Lys Val Ala Ile His Ala Glu Glu Glu Leu Ala
85 90 95

Ala Ala Arg Asp Ala Ala Ala Asp Ala Gln Arg Thr Gly Gln Arg Leu
100 105 110

Gln His Thr Val Thr Thr Leu Gln Arg Gln Leu Ala Ser Arg Glu Thr
115 120 125

Arg Arg Leu Ser Ala Ala Thr Ala Ile Gly Thr Asp Asp Leu Gly Gly
130 135 140

Gln Pro Gly Val Leu Phe Ala Glu Leu Phe Arg Arg Ala Asp Gln Arg
145 150 155 160

Ala Gly Glu Leu Ala Ala Tyr Ala Asp Arg Thr Arg Val Lys Trp Gln
165 170 175

Ala Cys Gly Arg Ala Tyr Gln Ala Ala Thr His Glu Ala Glu Lys
180 185 190

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2376 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: PROTEIN (SepA)

(ix) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

```
Met Arg Gln Asp Ile Met Tyr Asn Ile Asp Asp Ile Leu Glu Lys Val
 1               5                  10                  15

Asn Ala Pro Arg Ala Arg Leu Ser Glu Glu Asn Asp Thr Ala Val Thr
            20                  25                  30

Leu Thr Asp Leu Phe Ser Arg Ser Phe Pro Glu Val Lys Lys Ile Thr
        35                  40                  45

Gly Asp Ser Leu Ser Trp Gly Glu Val Cys Tyr Leu Tyr Ser Gln Ala
    50                  55                  60

Gln His Glu Gln Lys Glu Asn Arg Leu Thr Glu Ser Arg Ile Leu Ala
65                  70                  75                  80

Arg Ala Asn Pro Leu Leu Val Asn Ala Val Arg Leu Gly Ile Arg Gln
                85                  90                  95

Ala Ala Gly Ser Arg Ser Tyr Asp Asp Trp Phe Gly Ser Arg Ala Asp
            100                 105                 110

Arg Phe Ala Arg Pro Gly Ser Val Ala Ser Met Phe Ser Pro Ala Ala
        115                 120                 125

Tyr Leu Thr Glu Leu Tyr Arg Glu Ala Lys Asp Leu His Pro Asp Thr
    130                 135                 140

Ser Leu Phe Arg Leu Asp Ile Arg Arg Pro Asp Leu Ala Ala Leu Ala
145                 150                 155                 160

Leu Ser Gln Asn Asn Met Asp Asp Glu Leu Ser Thr Leu Ser Leu Ser
                165                 170                 175

Asn Glu Leu Leu Tyr Arg Gly Ile Gly Ala Ala Glu Gly Leu Asp Asp
            180                 185                 190
```

Asp Ser Val Arg Glu Leu Leu Ala Gly Tyr Arg Leu Thr Gly Leu Thr
195 200 205

Pro Tyr His Trp Ala Tyr Glu Ala Ala Arg Gln Ala Ile Leu Val Gln
210 215 220

Asp Pro Thr Leu Met Gly Phe Ser Arg Asn Pro Asp Val Ala Gln Leu
225 230 235 240

Met Asp Pro Ala Ser Met Leu Ala Ile Glu Ala Asp Ile Ser Pro Glu
245 250 255

Leu Tyr Gln Ile Leu Ala Glu Glu Ile Thr Thr Asp Ser Tyr Glu Ala
260 265 270

Leu Trp Ser Lys Asn Phe Gly Asp Met Pro Pro Ser Ser Leu Leu Ser
275 280 285

Tyr Asp Ala Leu Ala Thr Phe Tyr Asp Leu Asp Tyr Asp Glu Leu Thr
290 295 300

Ser Leu Leu Ser Leu Arg Leu Asp Phe Ser Asn Pro Asn Asn Glu Tyr
305 310 315 320

Tyr Ile Asn Ser Gln Leu Ser Val Val Thr Leu Asn Glu Ser Thr Gly
325 330 335

Leu Ile Thr Ile His His Tyr Leu Arg Thr Leu Gly Gly Asp Ser Gln
340 345 350

Gln Ile Asn Pro Glu Leu Ile Pro Tyr Gly Asp Gly Thr Tyr Leu Tyr
355 360 365

Asn Phe Ser Val Val Ser Thr Ile Ser Glu Asp Ser Phe Lys Leu Gly
370 375 380

Ser Leu Gly Ser Asn Ser Ser Asn Leu Tyr Ser Gly Asp Tyr Gln Leu
385 390 395 400

Gln Lys Gly Val Arg Tyr Ser Ile Pro Val Glu Ile Asp Glu Gly Lys
405 410 415

Leu Asn Asp Gly Ile Thr Ile Gly Leu Ser Arg Lys Gly Gly Gly Tyr
420 425 430

Tyr Ser Thr Val Asn Phe Thr Leu Ile Glu Tyr Asp Pro Ala Ile Phe
435 440 445

Ile Leu Lys Leu Asn Lys Val Ile Arg Leu Tyr Lys Ala Thr Gly Met
450 455 460

Thr Thr Ala Glu Ile Tyr Gln Ile Thr Asn Ile Leu Asn Asn Gly Leu
465 470 475 480

Thr Ile Asp His Ala Val Leu Ser Lys Ile Phe Leu Val Arg Tyr Leu
485 490 495

Met Arg His Tyr Gln Leu Asp Val Ala Arg Ser Leu Ile Leu Cys Asn

500 505 510

Gly Thr Ile Ser Asp Gln Ala Phe Ser Gly Glu Thr Gly Leu Phe Thr
515 520 525

Thr Leu Phe Asn Thr Pro Pro Leu Asn Gly Gln Leu Phe Ser Ala Asp
530 535 540

Asp Thr Pro Leu Asp Leu Arg Ser Glu Ala Pro Glu Asp Ala Phe Arg
545 550 555 560

Leu Ser Val Leu Lys Arg Ala Phe Asn Ile Ser Ala Ser Gly Leu Ser
565 570 575

Thr Leu Trp Gln Leu Ala Ser Gly Asp Ser Ser Ala Gly Phe Ser Cys
580 585 590

Ser Ala Asp Asn Ile Ala Ala Leu Tyr Arg Val Lys Leu Leu Ala Asp
595 600 605

Ile His Asp Leu Ser Ala Gly Glu Leu Ser Met Leu Leu Ser Val Ser
610 615 620

Pro Phe Ser Gly Val Ala Ala Gly Ser Leu Ser Asp Asn Glu Leu Thr
625 630 635 640

Gln Phe Leu Tyr Gln Thr Thr Thr Trp Leu Thr Glu Gln Gly Trp Thr
645 650 655

Val Ser Asp Val Phe Leu Met Leu Thr Thr Gln Tyr Gly Thr Leu Leu
660 665 670

Thr Pro Asp Ile Glu Asn Leu Leu Ala Ser Leu Arg Asn Gly Leu Ser
675 680 685

Gly Arg Glu Leu Phe Pro Glu Thr Leu Pro Gly Asp Gly Ala Pro Phe
690 695 700

Ile Ala Ala Ala Met Gln Leu Asp Ala Thr Asp Thr Ala Lys Ala Met
705 710 715 720

Leu Thr Trp Ala Asp Gln Leu Lys Pro Glu Gly Leu Thr Leu Thr Glu
725 730 735

Phe Ile Leu Leu Val Met Asn Ala Ala Pro Asn Asp Glu Gln Ala Gly
740 745 750

Gln Met Ala Gly Phe Cys Gln Ala Leu Trp Gln Leu Ala Leu Ile Ile
755 760 765

Arg Ser Thr Gly Leu Ser Thr Arg Glu Leu Thr Leu Leu Val Ser Gln
770 775 780

Pro Gly Arg Phe Arg Thr Gly Trp His His Leu Pro His Asp Leu Pro
785 790 795 800

Ala Leu Arg Asp Ile Thr Arg Phe His Ala Val Val Asn Arg Ser Gly
805 810 815

Ser His Ala Gly Glu Val Leu Thr Ala Leu Glu Thr Gly Glu Leu Ser
820 825 830

Ser Ala Leu Leu Ala Arg Ala Leu Ser Gln Asn Glu Gln Asp Val Thr
835 840 845

Gly Ala Leu Ala Gln Val Arg Gly Ala Gly Glu Gln Asp Asn Ser Val
850 855 860

Phe Thr Ser Trp Glu Glu Val Asp Gln Ala Glu Gln Trp Leu Asp Met
865 870 875 880

Ser Glu Thr Leu Ser Ile Thr Pro Ser Gly Leu Ala Ser Leu Ile Ala
885 890 895

Leu Lys Tyr Ile Asn Val Ser Asp Asp Ser Ala Pro Leu Tyr Ser Gln
900 905 910

Trp Gln Val Val Ser Gly Leu Leu Gln Ala Gly Leu Lys Ser Ser Gln
915 920 925

Ser Ser Ala Leu His Asp Tyr Leu Glu Glu Gly Thr Ser Ser Ala Leu
930 935 940

Cys Ala Tyr Tyr Leu Arg Asn Leu Ala Pro Asn Met Val Ser Gly Arg
945 950 955 960

Asp Asp Leu Phe Gly Tyr Leu Leu Leu Asp Asn Gln Val Ser Ala Lys
965 970 975

Val Lys Thr Thr Arg Ile Ala Glu Ala Ile Ala Gly Ile Arg Leu Tyr
980 985 990

Ile Asn Arg Ala Leu Asn Gly Ile Glu Leu Ser Ala Met Ala Glu Val
995 1000 1005

Arg Gly Arg Gln Phe Phe Thr Asp Trp Asp Thr Phe Asn Lys Arg Tyr
1010 1015 1020

Ser Thr Trp Ala Gly Val Ser Glu Leu Val Tyr Tyr Pro Glu Asn Tyr
025 1030 1035 1040

Leu Asp Pro Thr Val Arg Ile Gly Gln Thr Gly Met Met Asp Thr Leu
1045 1050 1055

Leu Gln Ser Val Ser Gln Ser Ser Ile Asn Arg Asp Thr Val Glu Asp
1060 1065 1070

Ala Phe Lys Thr Tyr Leu Thr Thr Phe Glu Gln Ile Ala Asn Leu Asn
1075 1080 1085

Thr Val Ser Gly Tyr His Asp Asn Ala Ser Met Thr Gln Gly Thr Thr
1090 1095 1100

Trp Tyr Val Gly Arg Ser Ile Thr Asp Gln Thr Asn Trp Tyr Trp Arg
105 1110 1115 1120

Ser Ala Asn His Ser Lys Ile Gln Asp Ser Met Met Pro Ala Asn Ala
1125 1130 1135

Trp Thr Gly Trp Thr Lys Ile Asn Cys Gly Met Asn Pro Trp Ser Asp
1140 1145 1150

Leu Val Cys Ser Val Phe Phe Asn Ser Arg Leu Tyr Val Val Trp Val
1155 1160 1165

Glu Glu Asn Gln Ser Ala Asp Thr Glu Ala Glu Ser Thr Thr Thr Thr
1170 1175 1180

Gln Gln Ser Tyr Thr Leu Lys Leu Ser Phe Arg Arg Tyr Asp Gly Thr
185 1190 1195 1200

Trp Ser Ser Pro Val Ser Phe Asp Ile Thr Gly Asn Ile Ala Phe Pro
1205 1210 1215

Glu Thr Gln Gly Met His Val Thr Cys Asn Pro Leu Thr Glu Gln Leu
1220 1225 1230

Tyr Cys Ala Phe Tyr Ser Val Thr Ser Lys Pro Asp Phe Asp Asn Ala
1235 1240 1245

Gln Leu Ile Ser Val Asp Asn Asp Met Thr Leu Asn Val Ile Ser Asp
1250 1255 1260

Ile Gly Ile Phe Lys Ser Val Ser His Glu Phe Asn Thr Ser Thr Glu
265 1270 1275 1280

Lys Phe Ile Asn Asn Val Phe Ser Asp Pro Ser Ala Asn Tyr Phe Val
1285 1290 1295

Ser Ala Thr Ser Leu Ile Asp Asp Val Ile His Ser Asp Phe Ser Leu
1300 1305 1310

Leu Asn Ser Lys Thr Thr Ser Thr Val Phe Thr Asn Glu Asp Ser Ser
1315 1320 1325

Leu Leu Thr Pro Glu Leu His Ile Thr Ala Asn Val Ser Cys Phe Val
1330 1335 1340

Ser Thr Ala Gly Ile Ala Thr Gln Ser Thr Ile Glu Lys Phe Val Gln
345 1350 1355 1360

Ala Gly Ile Glu Phe Glu Glu Ile Asn Phe Tyr Ala Gly Gln Ala Ala
1365 1370 1375

Gly Gly Phe Asp Gly Phe Val Gly Val Asp Val Ser Asn Ser Lys Val
1380 1385 1390

Tyr Gln Val Gly Lys Glu Ala Val Gly Val Thr Val Lys Ser Tyr Ser
1395 1400 1405

Val Thr Gly Val Ser Gly Ser Val Glu Leu Phe Ile Asp Ser Ser Asn
1410 1415 1420

Lys Tyr Phe Ser Gly Ile Leu Ser Asp Lys Met Ile Thr Ala Leu Ile

425 1430 1435 1440

Ser Gly Ser Thr Ser Lys Val Asn Tyr Val Ser Ser Ile Gly Ser Gln
1445 1450 1455

Asp Phe Trp Ser Val Lys Ser Leu Met Pro Ala Leu Gln Ile Tyr Glu
1460 1465 1470

Leu Ile Asp Asp Ile Ile Leu Thr Ser Gly Val Asn Gly Thr Glu Ile
1475 1480 1485

Lys Ser Trp Pro Ser Ala Glu Trp Tyr Asn Asp Lys Leu Ser Leu Gln
1490 1495 1500

Ser Gly Asn Asn Leu Phe Asn Thr Lys Ser Leu Ser Phe Thr Val Asn
505 1510 1515 1520

Thr Ser Asp Ile Val Glu Asp Glu Phe Asp Val Thr Phe Thr Phe Thr
1525 1530 1535

Ala Val Asp Gln Asn Asn Val Val Leu Ala Ala Arg Thr Ala Ile Leu
1540 1545 1550

Thr Val Ile Arg Asn Ile Asn Asn Asp Thr Ser Val Ile Ala Leu Arg
1555 1560 1565

Lys Asn Thr Arg Gly Ala Gln Tyr Ile Arg Phe Thr Ala Gly Asn Asp
1570 1575 1580

Val Ala Leu Ile Arg Leu Asn Thr Leu Phe Ala Arg Gln Leu Val Asp
585 1590 1595 1600

Arg Ala Asn Thr Gly Ile Asp Thr Ile Leu Ser Met Glu Thr Gln Arg
1605 1610 1615

Leu Thr Glu Pro Ala Leu Glu Glu Gly Ser Asp Val Phe Met Asp Phe
1620 1625 1630

Ser Gly Ala Asn Ala Leu Tyr Phe Trp Glu Leu Phe Tyr Tyr Thr Pro
1635 1640 1645

Met Met Val Phe Gln Arg Leu Leu Gln Glu Gln His Phe Pro Glu Ala
1650 1655 1660

Thr Arg Trp Leu Gln Tyr Val Trp Asn Pro Ala Gly His Val Val Asn
665 1670 1675 1680

Gly Val Leu Gln Asn Tyr Thr Trp Asn Val Arg Pro Leu Glu Glu Asp
1685 1690 1695

Thr Gly Trp Asn Asp Ser Pro Leu Asp Ser Ile Asp Pro Asp Ala Ile
1700 1705 1710

Ala Gln Tyr Asp Pro Met His Tyr Lys Val Ala Thr Phe Met Ser Tyr
1715 1720 1725

Leu Asp Leu Leu Ile Ala Arg Gly Asp Ala Ala Tyr Arg Leu Leu Glu
1730 1735 1740

Arg Asp Thr Leu Asn Glu Ala Arg Met Trp Tyr Val Gln Ala Leu Asn
745 1750 1755 1760

Leu Leu Gly Asp Glu Pro Tyr Ile Ser Phe Asp Ala Asp Trp Ser Ala
1765 1770 1775

Leu Thr Leu Gly Asp Ala Ala Ser Glu Val Thr Arg Arg Asp Tyr Gln
1780 1785 1790

Glu Ala Leu Leu Ala Val Arg Arg Leu Val Pro Ala Pro Glu Thr Arg
1795 1800 1805

Thr Ala Asn Ser Leu Thr Ala Leu Phe Leu Pro Gln Gln Asn Glu Val
1810 1815 1820

Leu Lys Gly Tyr Trp Gln Thr Leu Ala Gln Arg Leu His Asn Leu Arg
825 1830 1835 1840

His Asn Leu Ser Ile Asp Gly Gln Pro Leu Ser Leu Ser Val Tyr Ala
1845 1850 1855

Thr Pro Ser Glu Pro Ser Ala Leu Gln Ser Ala Val Val Asn Ser Ala
1860 1865 1870

Gln Gly Ala Ala Ala Leu Pro Ala Ala Val Met Pro Leu Tyr Ser Phe
1875 1880 1885

Pro Val Met Leu Glu Asn Ala Arg Gly Met Val Ser Leu Leu Thr Gly
1890 1895 1900

Phe Gly Asn Thr Leu Leu Gly Ile Thr Glu Arg Gln Asp Ala Glu Ala
905 1910 1915 1920

Leu Ala Lys Leu Leu Gln Thr Gln Gly Ser Glu Leu Ile Arg Gln Gly
1925 1930 1935

Leu Arg Gln Gln Asp Asn Val Leu Glu Glu Ile Asp Ala Asp Ile Ala
1940 1945 1950

Ala Leu Glu Glu Ser Arg Arg Gly Ala Gln Met Arg Phe Glu Arg Tyr
1955 1960 1965

Lys Val Leu Tyr Glu Ala Asp Val Asn Thr Gly Glu Lys Gln Ala Met
1970 1975 1980

Asp Leu Tyr Leu Ser Ser Ser Val Leu Ser Ala Ser Thr Ala Ala Leu
985 1990 1995 2000

Phe Leu Ala Glu Ala Ala Ala Asp Met Leu Pro Asn Ile Tyr Gly Leu
2005 2010 2015

Ala Val Gly Gly Ser Arg Tyr Gly Ala Leu Phe Lys Ala Thr Ala Ile
2020 2025 2030

Gly Ile Gln Val Ser Ser Asp Ala Thr Arg Ile Ser Ala Asp Lys Ile
2035 2040 2045

Ser Gln Ser Glu Val Tyr Arg Arg Arg Arg Glu Glu Trp Glu Ile Gln
2050 2055 2060

Arg Asp Ser Ala Gln Ser Asp Val Ala Gln Ile Asp Ala Gln Leu Ala
065 2070 2075 2080

Ala Met Ala Val Arg Arg Glu Gly Ala Glu Leu Gln Lys Thr Tyr Leu
2085 2090 2095

Glu Thr Gln Gln Thr Gln Ala Gln Ala Gln Leu Ala Phe Leu Gln Ser
2100 2105 2110

Lys Phe Asn Asn Thr Ala Leu Tyr Ser Trp Leu Arg Gly Arg Leu Ser
2115 2120 2125

Ala Ile Tyr Tyr Gln Phe Tyr Asp Leu Ala Val Ser Arg Cys Leu Met
2130 2135 2140

Ala Gln Gln Ala Trp Gln Trp Asp Lys Phe Glu Thr Arg Ser Phe Ile
145 2150 2155 2160

Gln Pro Gly Ala Trp Met Gly Ala Asn Ala Gly Leu Leu Ala Gly Glu
2165 2170 2175

Thr Leu Met Leu Asn Leu Ala Gln Met Glu Gln Ala Trp Leu Thr Gly
2180 2185 2190

Asp Glu Arg Ala Ile Glu Val Thr Arg Thr Val Cys Leu Ser Glu Val
2195 2200 2205

Tyr Thr Ser Leu Ala Glu Asp Ala Ala Phe Ser Leu Ala Asp Lys Val
2210 2215 2220

Val Glu Leu Val Ser Asn Gly Ser Gly Ser Ala Gly Thr Lys Ser Asn
225 2230 2235 2240

Gly Leu Gln Met Asp Gln Gln Gln Leu Glu Ala Thr Leu Lys Leu Ala
2245 2250 2255

Asp Leu Gly Ile Gly Asn Asp Tyr Pro Val Ser Leu Gly Thr Met Arg
2260 2265 2270

Arg Ile Lys Gln Ile Ser Val Thr Leu Pro Ala Leu Val Gly Pro Tyr
2275 2280 2285

Gln Asp Val Arg Ala Val Leu Ser Tyr Gly Gly Ser Met Val Met Pro
2290 2295 2300

Arg Gly Cys Ser Ala Leu Ala Val Ser His Gly Met Asn Asp Ser Gly
305 2310 2315 2320

Gln Phe Gln Leu Asp Phe Asn Asp Pro Arg Tyr Leu Pro Phe Glu Gly
2325 2330 2335

Leu Pro Val Asp Asp Thr Gly Thr Leu Thr Leu Ser Phe Pro Asp Ala
2340 2345 2350

Asp Gly Lys Gln Gln Ala Met Leu Leu Ser Leu Ser Asp Ile Ile Leu
2355 2360 2365

His Ile Arg Tyr Thr Ile Ile Ser
2370 2375

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1429 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: PROTEIN (SepB)

(ix) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

```
Met Gln Asn His Gln Asp Met Ala Ile Thr Ala Pro Thr Leu Pro Ser
 1               5                  10                  15

Gly Gly Gly Ala Val Thr Gly Leu Lys Gly Asp Ile Ala Ala Ala Gly
            20                  25                  30

Pro Asp Gly Ala Ala Thr Leu Ser Ile Pro Leu Pro Val Ser Pro Gly
        35                  40                  45

Arg Gly Tyr Ala Pro Thr Gly Ala Leu Asn Tyr His Ser Arg Ser Gly
    50                  55                  60
```

Asn Gly Pro Phe Gly Ile Gly Trp Gly Ile Gly Gly Ala Ala Val Gln
65 70 75 80

Arg Arg Thr Arg Asn Gly Ala Pro Thr Tyr Asp Asp Thr Asp Glu Phe
85 90 95

Thr Gly Pro Asp Gly Glu Val Leu Val Pro Ala Leu Thr Ala Ala Gly
100 105 110

Thr Gln Glu Ala Arg Gln Ala Thr Ser Leu Leu Gly Ile Asn Pro Gly
115 120 125

Gly Ser Phe Asn Val Gln Val Tyr Arg Ser Arg Thr Glu Gly Ser Leu
130 135 140

Ser Arg Leu Glu Arg Trp Leu Pro Ala Asp Glu Thr Glu Thr Glu Phe
145 150 155 160

Trp Val Leu Tyr Thr Pro Asp Gly Gln Val Ala Leu Leu Gly Arg Asn
165 170 175

Ala Gln Ala Arg Ile Ser Asn Pro Thr Ala Pro Thr Gln Thr Ala Val
180 185 190

Trp Leu Met Glu Ser Ser Val Ser Leu Thr Gly Glu Gln Met Tyr Tyr
195 200 205

Gln Tyr Arg Ala Glu Asp Asp Asp Gly Cys Asp Glu Ala Glu Arg Asp
210 215 220

Ala His Pro Gln Ala Gly Ala Gln Arg Tyr Pro Val Ala Val Trp Tyr
225 230 235 240

Gly Asn Arg Gln Ala Ala Arg Thr Leu Pro Ala Leu Val Ser Thr Pro
245 250 255

Ser Met Asp Ser Trp Leu Phe Ile Leu Val Phe Asp Tyr Gly Glu Arg
260 265 270

Ser Ser Val Leu Ser Glu Ala Pro Ala Trp Gln Thr Pro Gly Ser Gly
275 280 285

Glu Trp Leu Cys Arg Gln Asp Cys Phe Ser Gly Tyr Glu Phe Gly Phe
290 295 300

Asn Leu Arg Thr Arg Arg Leu Cys Arg Gln Val Leu Met Phe His Tyr
305 310 315 320

Leu Gly Val Leu Ala Gly Ser Ser Gly Ala Asn Asp Ala Pro Ala Leu
325 330 335

Ile Ser Arg Leu Leu Leu Asp Tyr Arg Glu Ser Pro Ser Leu Ser Leu
340 345 350

Leu Glu Asn Val His Gln Val Ala Tyr Glu Ser Asp Gly Thr Ser Cys
355 360 365

Ala Leu Pro Ala Leu Ala Leu Gly Trp Gln Thr Phe Thr Pro Pro Thr
370 375 380

Leu Ser Ala Trp Gln Thr Arg Asp Asp Met Gly Lys Leu Ser Leu Leu
385 390 395 400

Gln Pro Tyr Gln Leu Val Asp Leu Asn Gly Glu Gly Val Val Gly Ile
405 410 415

Leu Tyr Gln Asp Ser Gly Ala Trp Trp Tyr Arg Glu Pro Val Arg Gln
420 425 430

Ser Gly Asp Asp Pro Asp Ala Val Thr Trp Gly Ala Ala Ala Ala Leu
435 440 445

Pro Thr Met Pro Ala Leu His Asn Ser Gly Ile Leu Ala Asp Leu Asn
450 455 460

Gly Asp Gly Arg Leu Glu Trp Val Val Thr Ala Pro Gly Val Ala Gly
465 470 475 480

Met Tyr Asp Arg Thr Pro Gly Arg Asp Trp Leu His Phe Thr Pro Leu
485 490 495

Ser Ala Leu Pro Val Glu Tyr Ala His Pro Lys Ala Val Leu Ala Asp
500 505 510

Ile Leu Gly Ala Gly Leu Thr Asp Met Val Leu Ile Gly Pro Arg Ser
515 520 525

Val Arg Leu Tyr Ser Gly Lys Asn Asp Gly Trp Asn Lys Gly Glu Thr
530 535 540

Val Gln Gln Thr Glu Arg Leu Thr Leu Pro Val Pro Gly Val Asp Pro
545 550 555 560

Arg Thr Leu Val Ala Phe Ser Asp Met Ala Gly Ser Gly Gln Gln His
565 570 575

Leu Thr Glu Val Arg Ala Asn Gly Val Arg Tyr Trp Pro Asn Leu Gly
580 585 590

His Gly Arg Phe Gly Gln Pro Val Asn Ile Pro Gly Phe Ser Gln Ser
595 600 605

Val Thr Thr Phe Asn Pro Asp Gln Ile Leu Leu Ala Asp Thr Asp Gly
610 615 620

Ser Gly Thr Thr Asp Leu Ile Tyr Ala Met Ser Asp Arg Leu Val Ile
625 630 635 640

Tyr Phe Asn Gln Ser Gly Asn Tyr Phe Ala Glu Pro His Thr Leu Leu
645 650 655

Leu Pro Lys Gly Val Arg Tyr Asp Arg Thr Cys Ser Leu Gln Val Ala
660 665 670

Asp Ile Gln Gly Leu Gly Val Pro Ser Leu Leu Leu Thr Val Pro His

675 680 685

Val Ala Pro His His Trp Val Cys His Leu Ser Ala Asp Lys Pro Trp
690 695 700

Leu Leu Asn Gly Met Asn Asn Asn Met Gly Ala Arg His Ala Leu His
705 710 715 720

Tyr Arg Ser Ser Val Gln Phe Trp Leu Asp Glu Lys Ala Glu Ala Leu
725 730 735

Ala Ala Gly Ser Ser Pro Ala Cys Tyr Leu Pro Phe Thr Leu His Thr
740 745 750

Leu Trp Arg Ser Val Val Gln Asp Glu Ile Thr Gly Asn Arg Leu Val
755 760 765

Ser Asp Val Leu Tyr Arg His Gly Val Trp Asp Gly Gln Glu Arg Glu
770 775 780

Phe Arg Gly Phe Gly Phe Val Glu Ile Arg Asp Thr Asp Thr Leu Ala
785 790 795 800

Ser Gln Gly Thr Ala Thr Glu Leu Ser Met Pro Ser Val Ser Arg Asn
805 810 815

Trp Tyr Ala Thr Gly Val Pro Ala Val Asp Glu Arg Leu Pro Glu Thr
820 825 830

Tyr Trp Gln Asn Asp Ala Ala Ala Phe Ala Asp Phe Ala Thr Arg Phe
835 840 845

Thr Val Gly Ser Gly Glu Asp Glu Gln Thr Tyr Thr Pro Asp Asp Ser
850 855 860

Lys Thr Phe Trp Leu Gln Arg Ala Leu Lys Gly Ile Leu Leu Arg Ser
865 870 875 880

Glu Leu Tyr Gly Ala Asp Gly Ser Ser Gln Ala Asp Ile Pro Tyr Ser
885 890 895

Val Thr Glu Ser Arg Pro Gln Val Arg Leu Val Glu Ala Asn Gly Asp
900 905 910

Tyr Pro Val Val Trp Pro Met Gly Ala Glu Ser Arg Thr Ser Val Tyr
915 920 925

Glu Arg Tyr His Asn Asp Pro Gln Cys Gln Gln Gln Ala Val Leu Leu
930 935 940

Ser Asp Glu Tyr Gly Phe Pro Leu Arg Gln Val Ser Val Asn Tyr Pro
945 950 955 960

Arg Arg Pro Pro Ser Ala Asp Asn Pro Tyr Pro Ala Ser Leu Pro Ala
965 970 975

Thr Leu Phe Ala Asn Ser Tyr Asp Glu Gln Gln Gln Ile Leu Arg Leu
980 985 990

Gly Leu Gln Gln Ser Ser Ala His His Leu Val Ser Leu Ser Glu Gly
995 1000 1005

His Trp Leu Leu Gly Leu Ala Glu Ala Ser Arg Asp Asp Val Phe Thr
1010 1015 1020

Tyr Ser Ala Asp Asn Val Pro Glu Gly Gly Leu Thr Leu Glu His Leu
025 1030 1035 1040

Leu Ala Pro Glu Ser Leu Val Ser Asp Ser Gln Val Gly Thr Leu Ala
1045 1050 1055

Gly Gln Gln Gln Val Trp Tyr Leu Asp Ser Gln Asp Val Ala Thr Val
1060 1065 1070

Ala Ala Pro Pro Leu Pro Pro Lys Val Ala Phe Ile Glu Thr Ala Val
1075 1080 1085

Leu Asp Glu Gly Met Val Ser Ser Leu Ala Ala Tyr Ile Val Asp Glu
1090 1095 1100

His Leu Glu Gln Ala Gly Tyr Arg Gln Ser Gly Tyr Leu Phe Pro Arg
105 1110 1115 1120

Gly Arg Glu Ala Glu Gln Ala Leu Trp Thr Gln Cys Gln Gly Tyr Val
1125 1130 1135

Thr Tyr Ala Gly Ala Glu His Phe Trp Leu Pro Leu Ser Phe Arg Asp
1140 1145 1150

Ser Met Leu Thr Gly Pro Val Thr Val Thr Arg Asp Ala Tyr Asp Cys
1155 1160 1165

Val Ile Thr Gln Trp Gln Asp Ala Ala Gly Ile Val Thr Thr Ala Asp
1170 1175 1180

Tyr Asp Trp Arg Phe Leu Thr Pro Val Arg Val Thr Asp Pro Asn Asp
185 1190 1195 1200

Asn Leu Gln Ser Val Thr Leu Asp Ala Leu Gly Arg Val Thr Thr Leu
1205 1210 1215

Arg Phe Trp Gly Thr Glu Asn Gly Ile Ala Thr Gly Tyr Ser Asp Ala
1220 1225 1230

Thr Leu Ser Val Pro Asp Gly Ala Ala Ala Ala Leu Ala Leu Thr Ala
1235 1240 1245

Pro Leu Pro Val Ala Gln Cys Leu Val Tyr Val Thr Asp Ser Trp Gly
1250 1255 1260

Asp Asp Asp Asn Glu Lys Met Pro Pro His Val Val Val Leu Ala Thr
265 1270 1275 1280

Asp Arg Tyr Asp Ser Asp Thr Gly Gln Gln Val Arg Gln Gln Val Thr
1285 1290 1295

```
Phe Ser Asp Gly Phe Gly Arg Glu Leu Gln Ser Ala Thr Arg Gln Ala
                1300                1305                1310

Glu Gly Asn Ala Trp Gln Arg Gly Arg Asp Gly Lys Leu Val Thr Ala
            1315                1320                1325

Ser Asp Gly Leu Pro Val Thr Val Ala Thr Asn Phe Arg Trp Ala Val
        1330                1335                1340

Thr Gly Arg Ala Glu Tyr Asp Asn Lys Gly Leu Pro Val Arg Val Tyr
345                 1350                1355                1360

Gln Pro Tyr Phe Leu Asp Ser Trp Gln Tyr Val Ser Asp Asp Ser Ala
                1365                1370                1375

Arg Gln Asp Leu Tyr Ala Asp Thr His Phe Tyr Asp Pro Thr Ala Arg
            1380                1385                1390

Glu Trp Gln Val Ile Thr Ala Lys Gly Glu Arg Arg Gln Val Leu Tyr
        1395                1400                1405

Thr Pro Trp Phe Val Val Ser Glu Asp Glu Asn Asp Thr Val Gly Leu
    1410                1415                1420

Asn Asp Ala Ser
425
```

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 973 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: PROTEIN (SepC)

(ix) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

```
Met Ser Thr Ser Leu Phe Ser Ser Thr Pro Ser Val Ala Val Leu Asp
1               5                   10                  15

Asn Arg Gly Leu Leu Val Arg Glu Leu Gln Tyr Tyr Arg His Pro Asp
            20                  25                  30

Thr Pro Glu Glu Thr Asp Glu Arg Ile Thr Cys His Gln His Asp Glu
        35                  40                  45

Arg Gly Ser Leu Ser Gln Ser Ala Asp Pro Arg Leu His Ala Ala Gly
    50                  55                  60

Leu Thr Asn Phe Thr Tyr Leu Asn Ser Leu Thr Gly Thr Val Leu Gln
65                  70                  75                  80

Ser Val Ser Ala Asp Ala Gly Thr Ser Leu Glu Leu Ser Asp Ala Ala
                85                  90                  95

Gly Arg Ala Phe Leu Ala Val Thr Gly Ala Gly Thr Glu Asp Ala Val
            100                 105                 110

Thr Arg Thr Trp Gln Tyr Glu Asp Asp Thr Leu Pro Gly Arg Pro Leu
        115                 120                 125

Ser Ile Thr Glu Gln Val Thr Gly Glu Ala Ala Gln Ile Thr Glu Arg
    130                 135                 140

Phe Val Tyr Ala Gly Asn Thr Asp Ala Glu Lys Ile Leu Asn Leu Ala
145                 150                 155                 160

Gly Gln Cys Val Ser His Tyr Asp Thr Ala Gly Leu Val Gln Thr Asp
                165                 170                 175

Ser Ile Ala Leu Ser Gly Val Pro Leu Ala Val Thr Arg Gln Leu Leu
            180                 185                 190

Pro Asp Ala Ala Gly Ala Asn Trp Met Gly Glu Asp Ala Ser Ala Trp
        195                 200                 205

Asn Asp Leu Leu Asp Gly Glu Thr Phe Phe Thr Gln Thr His Ala Asp
    210                 215                 220

Ala Thr Gly Ala Val Leu Ser Ile Thr Asp Ala Lys Gly Asn Leu Gln
225                 230                 235                 240
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Arg Val Ala Tyr Asp Val Ala Gly Leu Leu Ser Gly Ser Trp Leu Thr
                245                 250                 255

Leu Lys Asp Gly Thr Glu Gln Val Ile Val Ala Ser Leu Thr Tyr Ser
            260                 265                 270

Ala Ala Gly Lys Lys Leu Arg Glu Glu His Gly Asn Gly Val Val Thr
        275                 280                 285

Ser Tyr Ile Tyr Glu Pro Glu Thr Gln Arg Leu Thr Gly Ile Lys Thr
    290                 295                 300

Glu Arg Pro Ser Gly His Val Ala Gly Ala Lys Val Leu Gln Asp Leu
305                 310                 315                 320

Arg Tyr Thr Tyr Asp Pro Val Gly Asn Val Leu Ser Val Asn Asn Asp
                325                 330                 335

Ala Glu Glu Thr Arg Phe Trp Arg Asn Gln Lys Val Val Pro Glu Asn
            340                 345                 350

Thr Tyr Ile Tyr Asp Ser Leu Tyr Gln Leu Val Ser Ala Thr Gly Arg
        355                 360                 365

Glu Met Ala Asn Ala Gly Gln Gln Gly Asn Asp Leu Pro Ser Ala Thr
    370                 375                 380

Ala Pro Leu Pro Thr Asp Ser Ser Ala Tyr Thr Asn Tyr Thr Arg Thr
385                 390                 395                 400

Tyr Arg Tyr Asp Arg Gly Gly Asn Leu Thr Gln Met Arg His Ser Ala
                405                 410                 415

Pro Ala Thr Asn Asn Asn Tyr Thr Thr Asp Ile Thr Val Ser Asp Arg
            420                 425                 430

Ser Asn Arg Ala Val Leu Ser Thr Leu Ala Glu Val Pro Ser Asp Val
        435                 440                 445

Asp Met Leu Phe Ser Ala Gly Gly His Gln Lys His Leu Gln Pro Gly
    450                 455                 460

Gln Ala Leu Val Trp Thr Pro Arg Gly Glu Leu Gln Lys Val Thr Pro
465                 470                 475                 480

Val Val Arg Asp Gly Gly Ala Asp Asp Ser Glu Ser Tyr Arg Tyr Asp
                485                 490                 495

Ala Gly Ser Gln Arg Ile Ile Lys Thr Gly Thr Arg Gln Thr Gly Asn
            500                 505                 510

Asn Val Gln Thr Gln Arg Val Val Tyr Leu Pro Gly Leu Glu Leu Arg
        515                 520                 525

Ile Met Ala Asn Gly Val Thr Glu Lys Glu Ser Leu Gln Val Ile Thr
    530                 535                 540

Val Gly Glu Ala Gly Arg Ala Gln Val Arg Val Leu His Trp Glu Ile
```

545 550 555 560

Gly Lys Pro Asp Asp Leu Asp Glu Asp Ser Val Arg Tyr Ser Tyr Asp
565 570 575

Asn Leu Val Gly Ser Ser Gln Leu Glu Leu Asp Arg Glu Gly Tyr Leu
580 585 590

Ile Ser Glu Glu Glu Phe Tyr Pro Tyr Gly Gly Thr Ala Val Leu Thr
595 600 605

Ala Arg Ser Glu Val Glu Ala Asp Tyr Lys Thr Ile Arg Tyr Ser Gly
610 615 620

Lys Glu Arg Asp Ala Thr Gly Leu Asp Tyr Tyr Gly Tyr Arg Tyr Tyr
625 630 635 640

Gln Pro Trp Ala Gly Arg Trp Leu Ser Thr Asp Pro Ala Gly Thr Val
645 650 655

Asp Gly Leu Asn Leu Phe Arg Met Val Arg Asn Asn Pro Val Thr Leu
660 665 670

Phe Asp Ser Asn Gly Arg Ile Ser Thr Gly Gln Glu Ala Arg Arg Leu
675 680 685

Val Gly Glu Ala Phe Val His Pro Leu His Met Pro Val Phe Glu Arg
690 695 700

Ile Ser Val Glu Arg Lys Ile Ser Met Ser Val Arg Glu Ala Gly Ile
705 710 715 720

Tyr Thr Ile Ser Ala Leu Gly Glu Gly Ala Ala Ala Lys Gly His Asn
725 730 735

Ile Leu Glu Lys Thr Ile Lys Pro Gly Ser Leu Lys Ala Ile Tyr Gly
740 745 750

Asp Lys Ala Glu Ser Ile Leu Gly Leu Ala Lys Arg Ser Gly Leu Val
755 760 765

Gly Arg Val Gly Gln Trp Asp Ala Ser Gly Val Arg Gly Ile Tyr Ala
770 775 780

His Asn Arg Pro Gly Gly Glu Asp Leu Val Tyr Pro Val Ser Leu Gln
785 790 795 800

Asn Thr Ser Ala Asn Glu Ile Val Asn Ala Trp Ile Lys Phe Lys Ile
805 810 815

Ile Thr Pro Tyr Thr Gly Asp Tyr Asp Met His Asp Ile Ile Lys Phe
820 825 830

Ser Asp Gly Lys Gly His Val Pro Thr Ala Glu Ser Ser Glu Glu Arg
835 840 845

Gly Val Lys Asp Leu Ile Asn Lys Gly Val Ala Glu Val Asp Pro Ser
850 855 860

```
Arg Pro Phe Glu Tyr Thr Ala Met Asn Val Ile Arg His Gly Pro Gln
865                 870                 875                 880

Val Asn Phe Val Pro Tyr Met Trp Glu His Glu His Asp Lys Val Val
                885                 890                 895

Asn Asp Asn Gly Tyr Leu Gly Val Val Ala Ser Pro Gly Pro Phe Pro
            900                 905                 910

Val Ala Met Val His Gln Gly Glu Trp Thr Val Phe Asp Asn Ser Glu
        915                 920                 925

Glu Leu Phe Asn Phe Tyr Lys Ser Thr Asn Thr Pro Leu Pro Glu His
    930                 935                 940

Trp Ser Gln Asp Phe Met Asp Arg Gly Lys Gly Ile Val Ala Thr Pro
945                 950                 955                 960

Arg His Ala Glu Leu Leu Asp Lys Arg Arg Val Met Tyr
                965                 970
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